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The Evolution of Early Life on Earth: An Analogue for Extraterrestrial Environments

Evolutionary Genomics Focus Group

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Evolutionary analysis of the genomes of organisms will help to build a clearer picture of how life originated, adapted to diverse environments, and increased in complexity on this planet. In turn, it will lead to a better understanding of life elsewhere in the Universe. This focus group includes astrobiologists from seven NAI Lead Institutions and several other institutions, combining expertise in molecular evolutionary analysis, organic chemistry and biochemistry, earth history, and paleontology. The unifying goal is to compare the early evolutionary history of life, as revealed through analyses of genomic sequence data, with changes in Earth's environment through time.

A web site (www.evogenomics.org) has been established for the focus group, providing contact information, publications, research activities, databases, and position announcements. Members of the focus group are: Steven A. Benner (U. Florida; benner@chem.ufl.edu), Jack D. Farmer (ASU; jfarmer@imap4.asu.edu), James R. Garey (U. South Florida; garey@chuma.cas.usf.edu), S. Blair Hedges (PSU; sbh1@psu.edu), Christopher H. House (PSU; chouse@geosc.psu.edu), David K. Jacobs (UCLA; djacobs@ucla.edu), Joseph L. Kirschvink (Caltech; kirschvink@caltech.edu), Sudhir Kumar (ASU; s.kumar@asu.edu), James A. Lake (UCLA; lake@mbi.ucla.edu), Charles R. Marshall (Harvard; marshall@eps.harvard.edu), Monica Riley (MBL; mriley@mbl.edu), Bruce N. Runnegar (UCLA; runnegar@ucla.edu), and Mitchell L. Sogin (MBL; sogin@evol5.mbl.edu).

Inter-team collaborations are underway in three research areas and are facilitated by monthly or bimonthly videoconferences and periodic workshops. (I) Phylogeny and Timescale. This is a research initiative (Animal Origins Project) that involves use of genomic tools to obtain large numbers of DNA sequences from representative animal phyla. These data will be used to estimate the phylogeny and timing of animal evolution to examine the influence of geologic and planetary-scale climatic events in the Neoproterozoic. (II) Gene Function. Research here includes documentation of recruitment in prokaryotic and eukaryotic proteins and development of computational tools to extract signals of form and function. (III) Horizontal Transfer. Analysis of complete genomes is made to understand the role of horizontal gene transfer in the evolution of life on Earth.

Protistan Microfossils in Early Mesoproterozoic Rocks

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The Roper Group, Northern Australia, comprises a thick (>1000 m), ramp-like succession of siliciclastic rocks deposited 1400-1500 million years ago in a rapidly subsiding intracratonic basin· The group can be divided into six principal depositional sequences bounded by conformable surfaces in the basin interior. Sequences are highly asymmetric, consisting mostly of highstand systems tracts dominated by deep basinal to distal shelf mudstones that shallow upward to coastal sandstones and intercalated shales. Poorly developed transgressive systems tracts consist of fluviatile to coastal marine redbeds overlain by shelf mudstones and thin, intercalated sand beds.

Fossils were first discovered in Roper rocks by Peat and others, who reported leiosphaerid acritarchs, small unicells, and filamentous microfossils in shales near the top of the succession. Our new study shows that microfossils are both abundant and unusually well preserved throughout the group. Thus, Roper rocks provide an unparalleled opportunity to study the distribution of Mesoproterozoic organisms in time and space.

The most abundant and diverse Roper assemblages come from estuarine to tide-dominated shoreline facies. Shales in lower shoreface to storm-dominated inner shelf facies preserve a lower abundance and diversity of acritarchs, as well as large striated tubes of uncertain, but probably eukaryotic, affinities. More distal shelf facies contain still fewer fossils; basinal mudstones contain much organic debris but few well-preserved microfossils. The observed onshore-offshore pattern of decreasing abundance, declining diversity, and changing dominance among fossils of probable eukaryotic origin is consistent with the hypothesis that trace elements critical to the biological N-cycle were sharply limited in Mesoproterozoic oceans. Under the hypothesized conditions, eukaryotic algae might well have been most abundant and diverse in estuarine settings, where Mo was most available.

The sole, but important exception to this distribution comes from shales deposited within the maximum flooding interval at the top of a deepening upward, transgressive systems tract. These samples contain distinctive acritarchs that bear one to twenty or more processes distributed asymmetrically about the vesicle surface. The processes vary in length within and among individuals, and some branch dichotomously. The irregular morphology and asymmetric distribution of these processes stand in marked contrast to the regular size and distribution of processes in most younger acanthomorphic acritarchs and suggest that the Roper fossils may have been actively growing cells or germinating cysts rather than metabolically inert vesicles. Bulbous protrusions on some specimens further suggest vegetative reproduction by budding. The kind of morphological remodeling necessary to form the morphologies represented in the Roper population requires a dynamic cytoskeleton, as well as sophisticated molecular signaling pathways for its regulation. Thus, by life’s middle age, eukaryotes had evolved the cytoskeletal architecture and regulatory networks that characterize living protists.

Roper paleontology indicates that 1500 million years ago, marine protists were cytologically sophisticated but environmentally limited. Roper fossils thus contribute to an emerging picture of Mesoproterozoic biology as transitional between an older world dominated by bacteria and archaea and a new world in which eukaryotes are also diverse and ecologically important.

Lithosphere-Hydrosphere Interactions
on the Hadean (>4.0 Ga) Earth

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Sources of information about environments for emergent life are provided by rare and scattered exposures of the oldest known granitoid gneisses and supracrustal enclaves, which are only between 3.8–4.0 Ga. The time period after primary accretion of the planets and the first appearance of a rock record has long been considered off-limits to geologists. Studies of the isotopic record of planetary evolution on Earth, the Moon and meteorites point to rapid planetary accretion, core formation [1] and the differentiation of some incompatible-element rich crust at an early stage [2]. To investigate conditions existing at the Earth's surface prior to 4 Ga requires discovery of yet older materials. Indeed, such ancient materials appear to have survived to the present. Early Archean (~3 Ga) quartzites of the Narryer Gneiss Complex in Western Australia host detrital zircon populations with ages >4 Ga [3,4] and new work has identified some of these as between 4.3 and 4.4 Ga, thereby establishing them as the oldest known terrestrial materials [5,6]. Trace element distributions [7,8] as well as oxygen isotope compositions [5,6,9] in zircon reflect the evolution and bulk geochemical characteristics of the parent melt. Recently reported *in situ* U-Pb and oxygen isotope results for the >4 Ga zircons from Western Australia have revealed details on the age and composition of their source magmas and represent the first steps toward directly exploring the nature of the Hadean crust.

Measurements of oxygen isotopes in rocks and minerals provide a means for understanding the magmatic, fluid and thermal history of the crust. Furthermore, oxygen isotopes are used to discriminate among possible granitoid sources (mantle, metasedimentary, hybrid) in accessory phases *e.g.*, igneous zircons. Crustal contamination can strongly affect oxygen isotope compositions in igneous rocks so that this signal becomes incorporated into crystallizing zircons. Zircon provides reliable U-Pb crystallization ages and can survive both high-grade metamorphism and sedimentary transport processes. Exchange rates for oxygen in zircon are very slow [10], thereby potentially permitting preservation of the protolith oxygen isotope composition even through high-grade metamorphism [9]. Granitoids derived largely from orthogneiss protoliths (so-called "I-types") tend to have δ^{18}O values below 9‰ whereas those derived by melting of clay-rich sedimentary rocks ("S-types") have δ^{18}O values that are higher. In general, granitoids considered as "S-type" form by melting of metasedimentary rocks enriched in ^{18}O; this is in contrast to "I-types" that form from melting of igneous rocks derived from arc processes. Since the average δ^{18}O values of Archean sedimentary rocks is >9‰, it requires only small inputs of this component to a primitive source magma to measurably raise its δ^{18}O from the canonical mantle value of +5.5 ‰. This mechanism can create "S-type" zircons in the rock record. Multi-collector ion microprobe measurements yield $\delta^{18}O_{SMOW}$ values of Hadean zircons ranging from +5.4±0.6‰ to

+15.4±0.4‰ [5]. The oxygen isotope fractionation between zircon and melt at magmatic temperatures is about -2‰ [9] allowing an estimate of the δ^{18} O value of the whole rock from which the zircon crystallized. Source δ^{18} O values calculated in this fashion for all zircon cores analyzed range from *ca.* 7.4 to 10.2‰. [5]. Based on these results, it is postulated that the *ca.* 4.3 Ga zircons formed from magmas containing a significant component of re-worked continental crust that formed in the presence of water near the Earth's surface. Mineral assemblages including muscovite and monazite suggestive of derivation from a peraluminous melt were found as inclusions in 4.2 Ga zircons from the Narryer Gneiss Complex [11]. Since the dominant mechanism to create peraluminous granitoids is melting of a graywacke protolith, this observation is also consistent with the presence of a hydrosphere on Earth prior to 4.2 Ga, that apparently was liquid and supported weathering and clay formation perhaps from emergent (continental?) landmasses. The formation of continental crust appears to be a feature unique to the geology of Earth. A sequence of events, beginning with water introduced to the shallow mantle by deep emplacement of hydrated oceanic crust and sediments, seems necessary for the production of granitoid melts in an arc environment. This process ultimately leads to the formation of continental crust.

What was this earliest terrestrial environment like? Could the "hydrosphere" have been present as a steam atmosphere as a result of a highly energetic early impact environment? It is generally thought that the early Earth experienced episodes of intensive bolide impacts in the time period 4400 to 4000 Ma. Yet, the lunar impact record appears to lack evidence of major impacts for most of this time aside from a peak in impact frequency centered at 3850 Ma associated with the late heavy bombardment [12,13]. These zircons are so ancient, some may be compelled to ask: Are they really terrestrial? Meteoritic zircons are very rare and REE and trace element compositions of these ancient zircons preclude a meteoritic origin [14,15]. If early impacts were important modifiers to the stability of an incipient surface biosphere during the Hadean, life may have emerged from protected environments *e.g.*, habitats in the crust or regolith or deep hydrothermal systems where the concentration and polymerization of biochemically important molecules could occur. In such subterranean nurseries for life, events at the surface may have had little consequence.

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Acknowledgments:

This work is being done in collaboration with T.M. Harrison (UCLA), R.T. Pidgeon (Curtin Institute of Technology, Perth, Western Australia) and C.D. Coath (UCLA) and is carried out at the National Ion Microprobe facility at UCLA, and the Astrobiology Materials Research Laboratory at CU-Boulder. On going studies are in collaboration with the Mission to Early Earth focus group within the NASA Astrobiology Institute.

The Evolution of O_2 and CO_2 in the Atmosphere

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A general consensus exists among geochemists on the evolutionary history of atmospheric CO_2: the pCO_2 level was considerably higher in the Archean, possibly 100-1000 PAL (the present atmospheric level), and gradually decreased with time to the present level. But at least three contrasting models currently exist for the evolutionary history of atmospheric O_2. The first model (e.g., Kasting, 1993) postulates the first major rise of pO_2 from an essentially oxygen-free level ($\sim 10^{-13}$ PAL) to ~0.15 PAL ca. 2 Ga ago, which was followed by a gradual increase to >0.5 PAL by ca ~600 Ma. The second model (e.g., Holland and Rye, 1999) postulates three step-wise rises of pO_2: the first rise from an essentially oxygen-free level to $\sim 10^{-3}$ PAL ca. 3 Ga ago, the second rise to ~0.15 PAL ca. 2 Ga, and the third rise to ~1 PAL ca. 600 Ma. The third model (e.g., Ohmoto, 1997) suggests the one major rise of pO_2, from $<10^{-4}$ PAL to ~1 PAL ca. 4 Ga ago. The deep (> ~500 m) ocean water becomes anoxic when the atmospheric pO_2 level is < ~0.5 PAL. Therefore, the first and second models of atmospheric O_2 evolution imply that the global ocean was anoxic until ~600 Ma ago. In contrast, the third model suggests that the redox structure of the ocean has been essentially the same since ~4 Ga. The connections between the change(?) of atmospheric pO_2 level and the emergences of major aerobic organisms (e. g., cyanobacteria, methanotrophs, eukaryotes), which occurred >2.7 Ga, are not well explained by any of the three models.

The geochemical data on Precambrian rocks that have been used to support the first and second models of atmospheric O_2 evolution include the following: (a) occurrences of banded iron formations (BIFs) in some >1.8 Ga marine sedimentary sequences; (b) occurrences of detrital grains of "unstable minerals" (uraninite, pyrite, and siderite) in some >2.4 Ga fluvial sediments; (c) occurrences of "Fe-depleted" rocks in some >2.0 Ga paleosols; (d) occurrences of <2.0 Ga redbeds; (e) large positive $\delta^{13}C$ values for some ~2.0 Ga carbonates; (f) small $\delta^{34}S$ variations among pyrite crystals in some >2.2 Ga marine sediments; (g) possible presence of mass-independent fractionation of ^{33}S and ^{36}S among sulfide and sulfates >2.0 Ga in age.

The controversy on the atmospheric O_2 evolution exists primarily because all the above geochemical data can also be explained by the third model. The third model presents the following additional lines of (bio)geochemical evidence: (a) occurrences of BIFs in modern oceans; (b-1) occurrences of the "unstable" detrital minerals in some modern sediments; (b-2) absence of "unstable" detrital minerals in "normal" sedimentary rocks of all ages; (c) occurrences of "Fe-depleted" rocks in soils of all ages; (c-2) occurrences of Fe^{3+} -enriched rocks in paleosols of all ages; (c-3) higher Fe^{3+}/Fe^{2+} ratios in sedimentary rocks, compared to igneous rocks, of all ages; (d) occurrence of some >2.2 Ga redbeds; (e) occurrences of carbonates with large $\delta^{13}C$ values in other geologic age; (f) large $\delta^{34}S$

variations for pyrite and barite in some >2.2 Ga sedimentary rocks; (g) possible presence of mass independent fractionation of ^{36}S among sulfide and sulfates of <2.0 Ga in age; (h) presence of Ce anomalies in BIFs of all ages; (i) biomarkers of eukaryotes in 2.7 Ga marine shales; (j) spatial (regional) and temporal (< 100 Ma) variations in $\delta^{13}C$ of organic matter in some >2.0 Ga marine sequences; (k) correlations between $\delta^{13}C$ of organic matter and $\delta^{34}S$ of pyrite in some Archean marine sequences; (l) similar contents of Mo, U, and V in shales of all ages; (m) similar contents of organic carbon and pyrite in shales of all ages; and (n) kerogen of cyanobacterial origin in 2.6 Ga paleosols.

Another approach to understand the evolution of atmospheric O_2 and CO_2 is from modeling of geochemical cycles. The atmospheric O_2 level has been controlled by the balance between the production flux, $F_{prod.O2}$ (= the burial flux of organic matter in marine sediments, $F_{b.org}$), and the consumption flux, $F_{sink.O2}$, which is composed of the consumption flux by reduced volcanic gas (e.g., H_2, CO, CH_4, H_2S), $F_{v.O2}$, and that by weathering of reduced compounds in rocks (organic carbon, sulfides, and FeO) during soil formation, $F_{s.O2}$. Presently, $F_{b.org} = F_{v.O2} + F_{s.O2}$. The first and second models of atmospheric O_2 evolution suggest very high fluxes of reduced volcanic gases prior to ~2.0 Ga, resulting in the relationships of $F_{b.org} < F_{v.O2}$, $pO_2 = \sim 0$, and $F_{s.O2} = \sim 0$. Our computer simulation of the coupled carbon-oxygen geochemical cycles, however, suggests that such a scenario would have resulted in nearly a complete conversion of CO_2 in the atmosphere and carbonates in the crust to organic carbon in less than 1 Ga since the emergence of cyanobacteria; this was an unlikely scenario. Under moderate fluxes of reduced volcanic gases, the pO_2 would have risen to ~1 PAL in less than 30 Ma since the emergence of cyanobacteria and the pO_2 level would have stayed at ~1 PAL over 3 Ga period while the atmospheric pCO_2 gradually decreased from ~1000 PAL to 1 PAL. Because this scenario also explains the present sizes of the carbonate and organic carbon reservoirs, it is a favorable one.

Geochronological Constraints on Early Animal Evolution and the Significance of the Precambrian-Cambrian Boundary

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The past five years have seen intensive efforts by paleontologists, evolutionary and developmental biologists, and geologists to better understand the first appearance and subsequent explosive diversification of animals. A high-precision U-Pb geochronological framework may be combined with paleontological, chemostratigraphic, and geological data to allow evaluation of models that invoke both intrinsic and extrinsic triggers for the Cambrian radiation.

Geochronological data from late Neoproterozoic rocks indicate Ediacaran fossils range from at least 575 Ma (Newfoundland) to 543 Ma (Namibia) and perhaps into the Cambrian. Complex trace-fossils occur at least as far back as 555 Ma (White Sea, Russia). The number and complexity of trace fossils increases dramatically to the Cambrian explosion which occurs over approximately a 10 Ma interval (530-520 Ma).

Critical to understanding the role of extrinsic factors in animal evolution is high-precision calibration of the global chemostratigraphic data. This will allow detailed evaluation of whether sudden isotopic excursions reflect globally synchronous events at the 100-300 ka level. Outstanding questions are: 1) what is the duration and number of late Neoproterozoic glacial events and their relationship to diversity in the fossil record? 2) is the negative $\delta^{13}C$ excursion at the Cambrian-Precambrian boundary globally synchronous and does it signal a major extinction and the creation of ecological niches for the ensuing Cambrian explosion?

New lithological, chemostratigraphic, biostratigraphic, and geochronological data from Oman constrain the timing and significance of events at the Precambrian-Cambrian boundary. The Ara Group (Huqf Supergroup) of the south Oman Salt Basin preserves the Cambrian/precambrian boundary and is endowed with numerous volcanic ash-beds. The boundary is defined by a prominent shift in carbon isotope values from +2 to –5 permil and a coincident enrichment in uranium. The fossil *Cloudina* occurs in rocks above and below the boundary. The age of the excurison is 542 ± 1 Ma which is synchronous with similar excursions on other continents and coupled with the uranium anomaly may reflect oxygen depletion in surface ocean water or perhaps the global ocean.

Continued integration of high-precision geochronology with biological and paleontological studies will allow new insights into the history of early animal evolution. We believe that this approach should allow discussion of the late Neoproterozoic-Cambrian record at the 100-300 ka level.

Pelobionts: Ancient Eukaryotes or Degenerate Protists?

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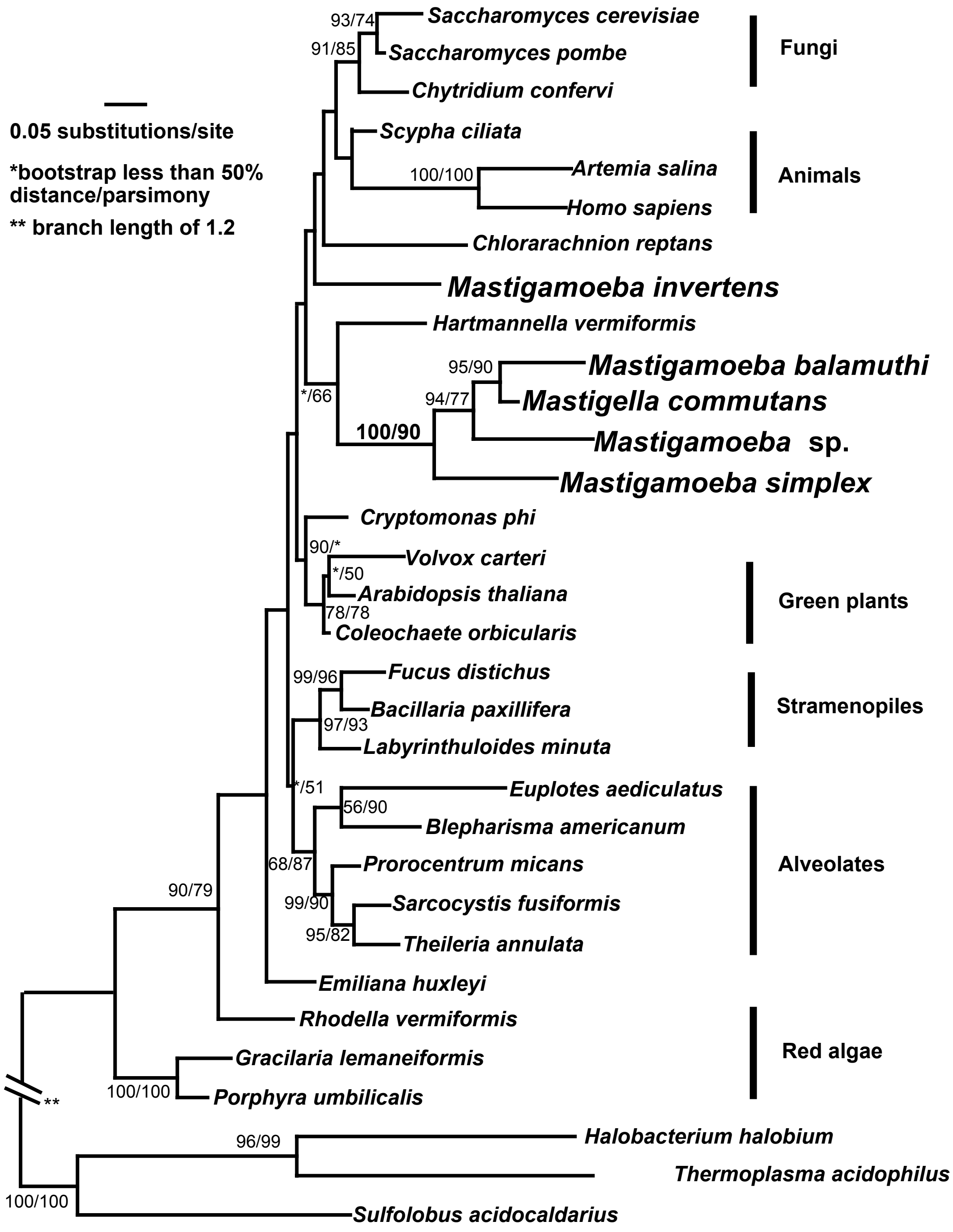
Phylogenetic analysis of small subunit ribosomal RNA (SSU rRNA) genes, including new pelobiont sequences from *Mastigella commutans, Mastigamoeba* sp., and *Mastigamoeba simplex*, forces us to re-evaluate some of our preconceived ideas about the early evolution of eukaryotes; namely the notion of ultrastructural simplicity as an ancestral trait. Based on cytoskeletal features, models of evolution in the early 1990's placed these free-living amoeboid flagellated protists close to the origins of the eukaryotic cell, in agreement with some early molecular phylogenies. Because of their potential significance to the understanding of the origins of early eukaryotes, sequences of the small subunit ribosomal RNA gene (SSU rRNA) were obtained from 3 additional pelobionts and subjected to a rigorous phylogenetic analysis. Despite the absence of typical eukaryotic organelles, e.g., mitochondria, dictyosomes, chloroplasts, coated vesicles, and peroxisomes, pelobionts are not basal to other eukaryotes in our molecular analyses nor are they specifically related to a previously described pelobiont-like organism, *Mastigamoeba invertens*. Many analyses suggest the pelobionts share common ancestry with the Entamoebidae, with *Endolimax nana* sharing an unique common evolutionary history with *Mastigamoeba simplex*. The reduction in cytoskeletal complexity may have occurred in a common ancestor of pelobionts and entamoebae, forcing us to re-evaluate our assumptions about what constitutes an ancestral eukaryotic trait.



Maximum likelihood analysis of eukaryotes based on comparison of SSU rRNA sequences. Bootstrap proportions are given as percentages near the individual nodes. The bootstrap values are computed under two different criteria: minimum evolution/maximum parsimony. Asterisks designate nodes with bootstrap values below 50%. Nodes with no bootstrap values noted were less than 50% under both criteria. Tree score is -lnL = 12048.422.

Considerations for Evaluation of Isotopic Evidence for Biological Activity

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Large isotopic fractionations have traditionally been earmarked as one of the hallmarks of biological activity. This is because biological processes can generate large magnitude isotopic fractionations in comparison to other geologically relevant fractionation processes. Atmospheric reactions have been known to be an exception to this rule and can generate some of the largest isotopic fractionations observed in nature, but they have generally been dismissed because they have not been thought capable of imprinting their character on minerals (and other phases) in rocks. Recent observation of mass-independent fractionations for sulfur and oxygen isotopes in samples of terrestrial rocks [1-3] and also of secondary minerals from SNC meteorites [4-7] presents several interesting considerations for the use of stable isotopes as probes for biological activity early in Earth history and on other planets. Here we discuss some of the considerations to be observed when using isotopes as probes of biological activity in environments where there is the potential for atmospheric imprints onto the isotope systematics observed in the rock record.

One of the unique characteristics of atmospheric isotope effects is that they can involve selection rules that are different from those traditionally considered in stable isotope geochemistry. For elements with more than two stable isotopes these isotopic fractionation effects are manifest as deviations from the traditional rules of mass-dependent fractionation (meaning that variations among $^{33}S/^{32}S$, $^{34}S/^{32}S$, and $^{36}S/^{32}S$ are related by the relative difference in mass between the isotope in the numerator relative to that in the denominator 1:2:4). Mass-independent fractionations of atmospheric origin are not required to obey the 1:2:4 relationships and therefore can provide a unique fingerprint of the action of atmospheric chemistry and atmosphere-surface exchange. Terrestrial ozone, for instance shows an enrichment of $^{17}O/^{16}O$ that is greater than predicted by the enrichment of $^{18}O/^{16}O$ for a mass-dependent process. This mass-independent fingerprint of atmospheric reactions makes it possible to identify their signature in the rock record and lead us to suggest that it will be important to consider as many isotope ratios as possible for a given element when evaluating fractionations of possible biological origin.

Another piece of information can be gained from high precision multiple isotope measurements is insight into the nature of the mass-dependent fractionation relationship among the different isotopes of a given element. The mass-dependent fractionation relationships for biological processes is slightly different than mass-dependent fractionation relationships produced by isotopic exchange reactions among rocks and minerals. The mass-dependence of many geological processes follow fractionation laws that are rooted in vibrational energy differences that impart a mass-dependence closer to $\delta^{33}S = 0.52\ \delta^{34}S$, whereas the mass-dependence of many biological processes also has diffusive terms that impart a slightly lower slope for mass-dependence. Below we present the results of an experiment that we have undertaken to determine the mass-dependent relationships for one biological fractionation processes —sulfur isotope fractionation associated with dissimilatory sulfate reduction by Archaeoglobus. The fractionation relationships yield $\delta^{33}S = 0.5117\ \delta^{34}S$ which is resolvable from the observed geological mass-fractionation relationship of $\delta^{33}S = 0.5155\ \delta^{34}S$ (figure 1). Our results imply that this type of information has the potential to be used to gain additional information about the fractionation process. Because biological processes often have diffusive components, another signature of their operation is resolvable with high-precision multiple isotope measurements. This presents us with another signature to be considered when applying multiple isotopes as probes of biological activity early in Earth's history, or on other planets.

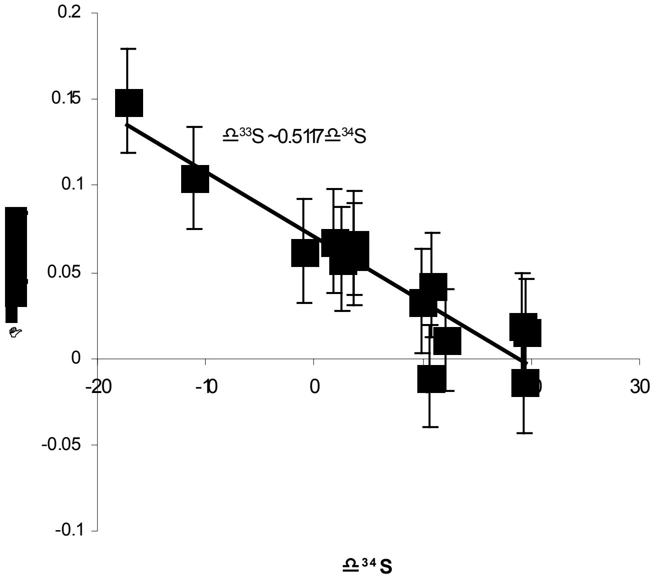

Figure 1 Plot of $\Delta^{33}S$ versus $\delta^{34}S$ for experiments conducted with Archaeoglobus. These data illustrate the difference in mass fractionation between those biological sulfate reduction under these conditions and those observed in the rock record. Error bars are 1σ. ($\Delta^{33}S = \delta^{34}S - 1000*((1+\delta^{34}S/1000)^{0.5155}-1)$)

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Deep Bilaterian Phylogeny: The Evolution of Adaptation to Extreme Environments

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Current studies of animal evolution based on a combination of molecular and morphological evidence and have shown that the bilateral animals are composed of three major groups: Deuterostomes such as echinoderms and vertebrates, Ecdysozoa, the molting protostomes, such as arthropods and nematodes and Lophotrochozoa, the non-molting protostomes such as annelids, molluscs and flatworms.

Adaptation to extreme environments has evolved in a number of ecdysozoan and lophotrochozoan phyla in a number of different ways. Some animals survive to extreme environments through symbiotic bacteria and other adaptations. The most well-known example are the animals associated with hydrothermal vents and cold seeps, such as vestimentiferan annelid and certain bivalve molluscs. These animals have adapted to hypersaline water rich in sulfide, methane and ammonia. Adult vestimentiferan worms have no mouth or gut and survive by symbiotic relationships with sulfur reducing and methanogenic bacteria contained within a large organ known as the trophosome.

Other animals have evolved the ability to survive extreme environments such as heat, cold, lack of food, hypoxia, dessication and even vacuum by the ability to form a resistant stage that allows the animal to survive until better conditions arrive. Tardigrades are a good example. Under harsh conditions or dessication, a tardigrade can transform into a tun in a matter of minutes. The tun stage is highly resistant to heat, freezing, dessication and vacuum. The tardigrade is completely inactive when a tun, so this is a mechanism to escape a harsh environment rather than the ability to actively live in a harsh environment. Some nematodes have evolved similar resistant stages as well. Under poor environmental conditions, a special resistant larval form known as a dauer will develop. Rotifers have solved the problem in a different way. When stressed, rotifers lay a special "resting egg" that is somewhat like a tun and is resistant to extreme environments. In this case, the survival of the offspring of the stressed animal is ensured, rather than the stressed animal itself.

Are there any metazoans that live actively in extreme environments without the aid of symbiotic bacteria? Many meiofaunal metazoans representing the phyla Platyhelminthes, Gastrotricha, Gnathostomulida, and Nematoda are good candidates. All of these groups have representatives that are associated with marine sediments that are rich in sulfide and low in oxygen. There have been reports of bacterial endosymbiosis in some species of nematodes and turbellarian flatworms that lack a mouth or gut (as in vestimentiferan worms) although in at least one nematode, the symbiotic bacteria are external rather than internal. However, many flatworms, gastrotrichs, gnathostomulids, and nematodes associated with sulfide rich anoxic sediment have complete digestive systems and appear to graze on bacteria in the sediment. Clearly, it will be important to focus on these animals in future studies.

The phylogenetic distribution of these animals suggests that the appearance of metazoans adapted to extreme environments has occurred many times by a variety of mechanisms including the adoption of bacterial endosymbionts, the development of sulfide detoxification systems, the evolution of anaerobic metabolism, the ability to undergo anhydrobiosis, the ability to form resistant larval forms and other as yet unknown mechanisms.

Environmental Conditions of the Early Earth at the Time of the Earliest Microfossil Record

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The 3.5 Ga Swaziland Supergroup, South Africa, contains some of the oldest known, and least metamorphosed sedimentary rocks on Earth. These rocks represent a preserved archive of early life, surficial processes, and environmental conditions on the early Earth. Chert is the most abundant sedimentary rock type in the volcanic part of this succession, the Onverwacht Group, and is widespread in the overlying Fig Tree Group. The cherts are enormously depleted in the heavy isotope of oxygen relative to younger cherts, and this has been interpreted as indicating very warm climatic temperatures, early oceans enormously depleted in heavy oxygen, or complete "resetting" of oxygen isotopes during later alteration. It now appears that the oxygen isotopic composition of the ocean is strongly controlled by circulation through hot mid-ocean ridges and could not have been significantly different in the past. Later "resetting" of the oxygen isotopes in cherts is an issue that cannot be settled by isotope geochemistry alone. We have therefore examined the isotopic variations in cherts within the context of their geologic setting to test all suggested alteration scenarios. We conclude below that altered cherts are readily recognizable and that the depletions in heavy oxygen relative to more recent cherts cannot be reasonably explained in terms of later "resetting". The isotopic data strongly indicate that climatic temperatures of the early Earth were on the order of 55°C–85°C and that the early evolution of life was a high temperature affair.

Cherts in the Swaziland Supergroup are silica replacements of a variety of pre-existing sediments that accumulated in various depositional environments. The complete spectrum of cherts was isotopically analysed and considered in terms of what was replaced, when it was replaced, and whether or not it was subjected to deformation and recrystallization. Cherts representing silicified volcanic materials have $\delta^{18}O$ indistinguishable from cross-cutting hydrothermal veins (+13‰ to +16‰). These low $\delta^{18}O$ values may result from the common presence of altered volcaniclastic impurities, especially micas, or they may indicate that these units formed via synsedimentary or later hydrothermal alteration. Silicified sediments range from +15‰ to +22‰ and occur as microquartz only in units younger than 3.445 Ga, the age of major regional deformation and magmatism. All older cherts consist of coarser quartz with $\delta^{18}O < +16‰$ and were probably recrystallized by the metamorphic event. Uplift and erosion at the end of Onverwacht time unroofed this isotopically stratified terrane to produce Swaziland conglomerates with chert clasts from

+15‰ to +22‰ overlain by Moodies clasts with δ^{18}O< +16‰. This inverse zonation demonstrates that chert δ^{18}O was fixed prior to Moodies time.

Significant later alteration from δ^{18}O-values initially higher than what is now observed is also precluded by the following:1) Where a single horizon can be traced into regions of higher metamorphic grade, there is no significant change in δ^{18}O. 2) Interbedded mafic/ultramafic igneous rocks are isotopically similar to Phanerozoic ophiolites indicating no later equilibration with the bedded cherts or wholesale lowering of δ^{18}O by influx of external fluids. 3) Coarse quartz replacing evaporite crystals is typically lighter than the matrix microquartz. Later alteration would preferentially affect the finer-grained microquartz. 4) White cherts in alternating black/ white layers are brecciated indicating earlier lithification than co-existing black cherts. The black cherts are up to 1.5‰ lighter and indicate crystallization at slightly deeper burial depths. Any later metamorphism did not eradicate these subtle differences.

The 6‰ isotopic variation of microquartz cherts is similar to that observed in Phanerozoic deep sea cherts where opal-A converts to microquartz during deep burial, but the actual Archean values are all 10‰ lower. The best explanation is that Onverwacht marine opal was converted to microquartz during shallow burial amidst numerous ground water convection cells associated with igneous activity that persisted throughout the depositional history. Cherts richest in ^{18}O formed in areas farthest removed from hydrothermal activity, areas of downward fluid entry, or at the extreme distal ends of exiting hydrothermal vents. The δ^{18}O variation is thus associated with lateral temperature variations of early burial rather than the vertical variations observed in deep sea Phanerozoic systems. The +22‰ maximum value indicates oceans that were either much warmer or strongly depleted in ^{18}O relative to modern values. Inasmuch as ^{18}O is apparently constrained to ±1‰ by sea floor basalt/water interactions, the alternative explanation is required. Temperatures of 55°- 85°C seem inescapable. The Archean ocean was **hot**!

Inasmuch as the Sun was apparently about 20% less luminous, the Earth at 3.5 Ga must have had substantial levels of greenhouse gases to be so much warmer than today. Only organisms tolerant of high temperatures could survive and evolve. This is compatible with interpretations of the microfossil record and is consistent with hyperthermophiles being deeply rooted in the RNA tree of life. Evolution of lower temperature organisms was retarded by the high climatic temperatures of the early Earth and had to await draw-down of greenhouse gases.

Oxygen solubility strongly decreases with increasing temperature and salinity. With ocean salinity possibly at 1.5 x– 2x the modern value, maximum dissolved O_2 in the ocean could have been only 2.5 ml/l even if the atmosphere contained 1 PAL O_2. The ocean was most likely hot, anoxic, and highly saline. Lower salinity water in estuaries and non-marine environments could have contained much higher dissolved O_2 or could have locally retained it better in the presence of photosynthesizing organisms. Such environments rather than the ocean may have been the sites of much early evolution.

Glacial Eustasy and the Flux of Iron from Midocean Ridge Hydrothermal Systems

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Midocean ridge hydrothermal systems support a diverse ecosystem on Earth, and perhaps on other planets or moons as well. A considerable body of experimental and observational evidence indicates, however, that the chemistry of these seawater-derived fluids is highly sensitive to the temperature and pressure at which they interact with the basalts of the seafloor, in part because the fluids tend to be close to the critical point of seawater. Thus, the particular fluid compositions that support life in the deep sea on Earth today may not have always been present on Earth or may not be present on other planetary bodies.

In experimental simulations of seafloor hydrothermal systems, pH falls and both Fe^{2+} and H_2S concentrations increase with increasing temperature, but the ratio Fe^{2+}/H_2S increases as well. This fact has fueled speculation that higher temperatures of water-rock interaction in the Archean may have promoted the establishment of Fe^{2+}-rich oceans that supported widespread banded iron formation. Less discussed is the pressure dependence of the Fe^{2+} concentration.

Depressurization of hydrothermal fluids in contact with basalt at elevated temperatures (350-425°C) causes substantial increases in Fe^{2+} concentrations, primarily as a result of increased stability of ferrous-chloride aqueous species and decrease in mineral-buffered pH at lower pressures. This observation leads us to speculate that glacioeustatic sea-level fluctuations may have caused variations in iron supply to the oceans, both on Quaternary time scales (~10 bar fluctuations) and at times in the more distant past, e.g., during the Neoproterozoic Snowball Earth episodes, when continental ice sheets may have been considerably larger than those of the Quaternary. The higher fertility of the Quaternary glacial ocean, and the notable association of Neoproterozoic glacial deposits and iron formation, may be at least in part a consequence of this phenomenon.

There are complications, however, including the consequences of phase separation and cooling of the fluid upon ascent from near the magma chamber to the seafloor. The significant variability in Cl^- concentrations of some modern vent fluids indicates that phase separation does occur; the low Fe^{2+} concentration of these fluids indicates that aqueous iron partitions nearly completely into the residual brine. The ultimate fate of this metal-rich brine is unknown, but it may not immediately vent to the overlying ocean. Cooling of vent fluid occurs upon ascent by conduction through wall rocks or mixing with cold seawater. A consequence of this cooling is the precipitation of iron-bearing minerals, particularly pyrite but also epidote and chlorite, and thus the reduction in Fe concentration of the fluid. These complications make unambiguous calculations of variations of iron flux problematic. Although these complications may affect the rate of Fe delivery to seawater, it is still likely that even subtle changes in pressure in subseafloor reaction zones at high temperatures, could have the potential for creating significant reservoirs of Fe-bearing fluids with implications for attendant impact on the paleo-marine environment and its sedimentary record.

Biogeochemistry of the 1640 Ma McArthur River (HYC) Lead-Zinc Ore and Host Sediments, Northern Territory, Australia

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The 1640 Ma McArthur River (HYC) lead-zinc-silver deposit of northern Australia is the largest known stratiform base-metal deposit. It formed in a hydrothermal system. It is extraordinarily well preserved, with all indicators of thermal maturity suggesting that the country rocks have not been heated beyond the 'oil window'. Oil bleeds from drill cores from nearby indicate petroleum source rocks have generated oil close to HYC. The deposit has been intersected by more than 100 drill holes, and is exposed in an underground mine. It represents a superb opportunity to examine this class of ore deposits, and consequently has been intensively studied. It also represents an extraordinary opportunity to study the paleobiology of an ancient hydrothermal system.

Interpretations of the genesis of the ore vary widely, with there being three end-member models: syngenetic (exhalative) in a deep marine setting; early diagenetic in the mud and silt of an ephemeral lake or sabkha; and early diagenetic in a marine environment below wave-base. Our work supports the last of these, as do recent sedimentological studies.

We have analysed hydrocarbon biomarkers from the ore and associated sediments sampled on a centimetre to millimetre scale. We have also analysed the $\delta^{13}C$ composition of selected hydrocarbons to help determine their biological affinity. Biomarker data suggest a marine environment of deposition and are consistent with data previously collected from the host Barney Creek Formation in the adjacent Glyde Sub-basin. An unusual biomarker distribution found in some samples from within 2-orebody is considered to be related to the former presence of sulfide oxidizing bacteria. These organisms flourished after turbidite deposition, when oxygen in the upper part of the water column was mixed down to the sediment water interface. The biomarker data are supported by micropaleontological observations on microfossils from the same samples and are consistent with intermittent oxygenation of the water column to the sediment - water interface. This observation suggests an extension of the known occurrence of sulfide oxidizing bacteria back in time by 800 million years, to 1640 Ma.

We have also completed a study of aromatic hydrocarbons to provide thermal history information. It is possible to reconstruct the original fluid flow patterns and to determine thermal gradients. Absolute temperatures are more difficult to determine, but are likely to range from ambient seafloor temperatures up to about 150^0C or more.

This work is continuing. Our plan is to determine as much of the paleobiology and paleoecology of this deposit as is possible with current techniques.

*Speaker

Carbon Dioxide Cycling and the Climate of Ancient Earth

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The continental cycle of silicate weathering and metamorphism dynamically buffers atmospheric CO_2 and climate. Feedback is provided by the strong temperature dependence of silicate weathering. Here we argue that hydrothermal alteration of oceanic basalts also dynamically buffers CO_2. The oceanic cycle links with the mantle via subduction and the midocean ridges. Feedback is provided by the dependence of carbonatization on dissolved carbonates in seawater. Unlike the continental cycle, the oceanic cycle has no thermostat. Currently the continental cycle is more important, but earlier in Earth's history, especially if heat flow were higher than it is now, more vigorous plate tectonics would have made the oceanic cycle dominant. We find that CO_2 greenhouses thick enough to defeat the faint early sun are implausible and that, if no other greenhouse gases are invoked, very cold climates are expected for much of the Proterozoic and the Archean. We echo current fashion and favor biogenic methane as the chief supplement to CO_2. Fast weathering and probable subduction of abundant impact ejecta would have reduced CO_2 levels still further in the Hadean. Despite its name, the Hadean would have been the coldest era in the history of the Earth.

A Model for Evolution of the Seawater Oxygen Isotope Composition

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Several well-argued interpretations of oxygen isotope data from apparently well-preserved marine carbonate components, as well as interpretations of the $\delta^{18}O$ of chert, require substantial changes in the oxygen-isotopic composition of seawater (δ_w) through time. However, most workers have concluded that such changes are unlikely because δ_w is so strongly buffered by high-temperature alteration of basalts at the mid-ocean ridge. In light of the fundamental disagreement between the data and models we have constructed a steady-state, time-dependent model for seawater δ_w using the latest analyses of fluxes and isotopic fractionations that influence seawater δ_w. We have conducted sensitivity tests of the steady-state model, and have driven the preferred forward model with a combination of parameters that are judged to be most important in forcing system changes. In particular, we vary the seafloor basalt production rate and the global silicate weathering rate in accordance with values derived from independent geochemical cycle models for the Phanerozoic and with fewer constraints from the Precambrian. We also explore the early evolution of δ_w in response to the growth of continents and the sedimentary shell.

Our suite of simulations leads us to conclude that, using reasonable ranges of values for rates of creation of ocean crust and continental weathering, major changes in the δ_w of seawater during the Phanerozoic are unlikely. This result is not new, inasmuch as other workers have come to the same conclusion using different model formulations and assumed fluxes This result is also in accord with studies of the oxygen isotope profiles through ancient ophiolites of various ages, which can be interpreted in terms of little or no change in the δ_w of seawater (0 ± 2 permil) from the Archean to present. Our understanding of oxygen mass and isotopic fluxes in the present Earth system is reasonably good. The models can tolerate some relatively large changes to the assumed values with no major consequences to our conclusion that seawater δ_w has experienced only minor variations through most of the Precambrian and Phanerozoic as the result of tectonic and sedimentary processes. The short- and long-term changes in $\delta^{18}O$ seen in carefully screened marine carbonate $\delta^{18}O$ records require interpretations in terms of other processes. We would like to remain optimistic regarding the utility of stable isotopes for reconstructions of the past, despite the skepticism that has been expressed in favor of virtually all carbonates having been altered in some way. We presently favor either ice-volume induced variations in the global δ_w or more local to interbasinal evaporation and runoff differences as a cause of the observed oxygen isotopic variability on scales of 10^6 to 10^8 yrs. Although a tremendous effort has been expended in collecting and culling samples to date, our present understanding of the record must be tempered by an appreciation for the sampling bias that must be inherent in any compilation of stable isotopic data.

Ecophysiological Changes in Microbial Mats Incubated in a Greenhouse Collaboratory

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Microbial mats are modern examples of the earliest microbial communities known. Among the best studied are microbial mats growing in hypersaline ponds managed for the production of salt by Exportadora de Sal, S.A. de C.V., Guerrero Negro, Baja

California Sur, Mexico. In May, 2001, we collected mats from Ponds 4 and 5 in this system and returned them to Ames Research Center, where they have been maintained for a period of over 9 months. We report here on both the ecophysiological changes occurring in the mats over that period of time as well as the facility in which they were incubated. Mats (approximately 1 sq. meter total area) were incubated in a greenhouse facility modified to provide the mats with natural levels of visible and ultraviolet radiation as well as constantly flowing, temperature-controlled water. Two replicated treatments were maintained, a “high salinity” treatment (about 120 ppt) and a “low salinity” treatment (about 90 ppt). Rates of net biological activity (e.g. photosynthesis, respiration, trace gas production) in the mats were relatively constant over the several months, and were similar to rates of activity measured in the field. However, over the course of the incubation, mats in both treatments changed in physical appearance. The most obvious change was that mats in the higher salinity treatments developed a higher proportion of carotenoid pigments (relative to chlorophyll), resulting in a noticeably orange color in the high salinity mats. This trend is also seen in the natural salinity gradient present at the field site. Changes in the community composition of the mats, as assayed by denaturing gradient gel electrophoresis (DGGE), as well as biomarker compounds produced in the mats were also monitored. The degree to which the mats kept in the greenhouse changed from the originally collected mats, as well as differences between high and low salinity mats will be discussed.

The greenhouse facility at Ames Research Center is being developed as a collaboratory testbed for research on agent-assisted remote scientific experimentation. This testbed is designed to enable a geographically distributed group of scientists to operate scientific equipment, take experimental measurements, share results, and collaborate in real time with remote colleagues. We plan to build intelligent agents to assist in the conduct of microbial mat experiments. The agents will function as lab assistants that initiate, monitor, and supervise experiment execution with the involvement of scientists as needed. As part of this effort, information on the interaction between scientists and agent-assistants will be collected and analyzed to develop collaboratory design recommendations for future NASA mission settings. As part of our initial hardware development to support the collaboratory, we have constructed an X,Y,Z positioning table over the mats which is capable of automatically positioning sophisticated instruments (including microelectrodes) at any location in the mats. This positioning system, and the instrumentation package is viewable over the internet (http://greenhouse.arc.nasa.gov) via a webcam hooked up to a computer located in the greenhouse.

We are making strides to determine what, if any, changes to microbial mats occur simply as a result of our manipulations and long-term incubations. The ability to perform meaningful manipulations of intact microbial communities is an important capability to develop in order to address changes that have occurred on Earth over geologic time. In future experiments we will address not only natural variations in environmental parameters, such as salinity, but periods of Earth’s history no longer available for direct measurement, such as low water sulfate concentrations and low oxygen tensions.

The Evolutionary Position of Nematodes

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The complete genomes of three animals (metazoans) have been sequenced by global research efforts: a nematode (*Caenorhabditis elegans*; Phylum Nematoda), an insect (*Drosophila melanogaster*; Arthropoda), and a vertebrate (*Homo sapiens*; Vertebrata). Remarkably, the relationships of these three species have yet to be clarified, primarily because of confusion surrounding the position of nematodes. Traditional morphology-based phylogenies place the pseudocoelomate nematodes and the acoelomate platyhelminthes basal to other bilateral animals. Those phylogenies also place arthropods and annelids together in the clade Articulata, based on the shared characteristic of segmentation. A new arrangement for metazoans was recently proposed based on 18S rDNA sequence data. In this molecular-based phylogeny, nematodes occupy a higher position in the tree, grouping with arthropods and other molting animals in a clade termed Ecdysozoa (based on the shared characteristic of ecdysis). However, only a slow-evolving nematode (*Trichinella*, Trichocephalida) generated this clade; inclusion of faster evolving species (e.g. *Caenorhabditis*, Rhabditida) pulled the nematodes back into a basal position. Further analysis of 18S sequence data has both supported and failed to support the Ecdysozoa clade. Also, a recent analysis of 50 nuclear proteins failed to support Ecdysozoa. Resolving this question will facilitate more efficient use of genomic data for animal evolution and genetics.

To examine the support for an Ecdysozoan clade and investigate the placement of nematodes within the Metazoa, we have analyzed over one hundred protein sequence quartets (Chordate, Arthropod, Nematode, Outgroup) and assessed molecular phylogenies from individual and combined protein alignments. To examine the potential bias in the rate of evolution within Nematoda, quartets were assembled with the distantly related *Caenorhabditis elegans* and *Brugia malayi* (Spirurida). Our results show that regardless of the species used to represent Nematoda, phylogenetic support for the basal position of nematodes is high with slow-evolving proteins, but decreases as evolutionary distance increases. This trend is observed regardless of the measure of evolutionary distance. Analysis of rate-constant proteins also shows this trend. To examine a possible taxon-sampling bias, we increased the number of phyla represented in a subset of protein phylogenies; tree topology proportions remain consistent with those found in the two quartet analyses and the rate-constant protein analysis. Our findings suggest that the placement of nematodes within the Metazoa is enigmatic, but that a basal position is supported with a high level of confidence with a large protein data set.

Detection of Morphological Fossils of Microbial Communities at Life's Upper Temperature Limit: An Astrobiological Search Strategy for Evidence of Biofilms

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Introduction

Biogeochemical interactions in high-temperature (>80°C) ecosystems produce microbial biosignatures, any number of which could become preserved in the rock record. Geomicrobiological studies in modern silica-depositing hydrothermal ecosystems (e.g., hot springs) demonstrate the variety of microbial biosignatures that can form. These include microbial fossils, microbially influenced accretionary growth structures (e.g., biogenic stromatolites), organic biomarker compounds, biologically fractionated isotopic signatures of organic compounds and minerals, and minerals whose microstructure or morphology were influenced by microbes. Paleobiological studies of hydrothermally precipitated chert deposits and hydrothermally silicified sediments have shown that relicts of these microbial biosignatures, albeit highly altered, are preserved even in Archean rocks.

Since ancient hyperthermophilic biosignatures are significantly altered, deciphering paleobiological and paleoenvironmental information from them requires an understanding of how such biosignatures formed and were altered by taphonomic processes and diagenetic mineral transformations. An iterative approach to the problem, the one taken here, involves comparing siliceous sinters formed in modern ecosystems occupied by hyperthermophilic communities, hydrothermal sinters produced abiotically, and hydrothermal cherts preserved in older analog deposits. Four key components of this strategy include: (1) characterizing the specimens before and after they are collected from the field across a range of spatial scales (meter to submicrometer), (2) identifying the characteristics of morphological fossils that were produced by abiogenic (chemical and physical/mechanical) processes, as well as those produced by biological processes, (3) determining whether the various biogenic components of a structure can be attributed to the presence of a specific biological community or dominant member of the population, and (4) describing quantitatively the various physical, chemical and biological processes that lead to the formation and post-depositional alteration of the structures.

Biogenic Stromatolites

Microbial communities play an important role in defining and modifying the surface characteristics of accretionary structures, and siliceous hydrothermal precipitates are no exception [1, 2]. Microbial communities dominated by phototrophs form thick (mm-cm) microbial mats and occupy the ambient to medium temperature regions (<80°C) of hot spring outflow channels and runoff surfaces. Chemolithotrophic communities occur as thin biofilms that colonize the surfaces of geyserite, the high temperature form of siliceous sinter. The sequence of biological and abiological events that led to the formation of geyserite deposits were documented to elucidate the role microorganisms play in geyserite morphogenesis. The morphology and microstructure of spicular, columnar, pseudocolumnar, and stratiform geyserite differ. Hyperthermophilic communities, dominated by distinctive, filamentous morphotypes, provide a continuous supply of colloidal-size substrates for the precipitation and adsorption of opaline silica. The distribution of the primary colonizers on the accretionary surfaces of the geyserites imposes the most significant control on the subsequent development of geyserite microstructures.

Microbial Fossils

The structural and chemical fidelity of fossilized microbial cells depend upon the sequence of biogeochemical interactions that lead to their formation. The various stages of microfossil formation in modern ecosystems were documented to study whether bona fide (permineralized and non-mineralized carbonaceous [3]) microfossils were produced. Preservation was found to depend upon the intrinsic characteristics of the microorganisms, the extrinsic characteristics of their environment, and the relative timing of mineral precipitation vs. microbial degradation. Diagenetic mineral transformations alter siliceous microfossils and the fine-grained opaline silica matrix in which they are preserved, thereby affecting the structural and chemical fidelity of morphological fossils, especially during the earliest stages of mineral diagenesis before opaline silica phases transform to microfibrous and microcrystalline varieties of quartz.

Conclusions

Ancient hydrothermal deposits represent potential paleobiological repositories on Earth and on other planetary bodies. Knowing how biosignatures of chemolithotrophic communities living near life's upper temperature limit are preserved improves our ability to interpret how microbial communities have interacted with their environments throughout geological time. Modern analog study of the biogeochemical interactions that lead to the preservation of biosignatures produced by microbial biofilms improves our ability to detect and properly interpret them.

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Biogenic Methane and the Rise of Oxygen

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Oxygenic photosynthesis does not make the rise of oxygen inevitable. What is required is that reductant and oxygen be separated and permanently segregated. The usual picture for Earth is that oxygenic photosynthesis split CO_2 into carbon and oxygen, with the carbon buried in sediments and the oxygen mostly taken up by oxides of iron and sulfur. The relatively small atmospheric reservoir of O_2 is regulated by the carbon burial rate, reaction with volcanic and metamorphic gases, and oxidation of reduced carbon released as old sediments weather. Absent from this picture is a distinction between the Archean and modern times: on average, carbon burial fluxes would have been matched by oxygen losses then as now [1]. Separation of reductant from oxidant is only provisional. No net oxidation of the continents occurs, and so no change of diagenetic, metamorphic, or volcanic gases is expected. Nor would any change in oxidative weathering be expected. Something more than carbon burial is required to make the Archean different.

The escape of hydrogen to space permanently separates the reductant from the oxidant. Hydrogen escape is widely believed to have led to the present highly oxidized states of Mars and Venus. Hydrogen escape has usually been thought small for Archaean Earth, because water vapor is cold-trapped at the troposphere and thus held to levels of a few ppmv in the stratosphere. This cold trapping renders hydrogen escape negligible. However, methane is not cold trapped, and its expected abundance in the Archaean, given low oxygen levels and a biogenic source, would have been high, probably more than 100 times present. At such levels methane would have driven geologically significant levels of hydrogen escape.

Oxygenic photosynthesis can be summarized by the overall reaction:

$$CO_2 + H_2O + \text{light} \rightarrow CH_2O + O_2$$

Methanogensis can be summarized by the overall reaction:

$$2CH_2O \rightarrow CH_4 + CO_2$$

The net result of methanogenesis is the production of free oxygen:

$$CO_2 + 2H_2O + \text{more light} \rightarrow CH_4 + 2O_2.$$

When hydrogen is lost to space via methane, oxygen is irreversibly gained:

$$CH_4 + 2O_2 \rightarrow CO_2 + O_2 + 4H(\text{space}).$$

In the Archean, this net gain of oxygen would have been rapidly sequestered into crustal sinks. Photochemical models of anoxic atmospheres predict methane levels ~200-3000 ppmv [2,3]. These levels are high enough to be climatologically significant. Limiting flux acting on 200-3000 ppmv of CH_4 then predicts Earth's oxidation at rates of order 1-15 Tmoles/yr O2 equivalents. These rates are comparable to the modern carbon burial rate of 10 Tmoles/yr O2 equivalent.

We suggest that time-integrated oxidation due to methane-induced hydrogen escape may have led to the rise of oxygen. Hydrogen buried in space cannot be mixed back into the crust. It is gone. We suggest that as the crust became generally more oxidized, oxidative reactions consuming O_2 required ever higher amounts of O_2 to occur, in accord with LeChatelier's principle. (In detail, much of this oxidation would have been of iron in basalts, the largest reservoir of oxidant in the Earth's continental crust [4], probably via sulfates in hydrothermal fluids). Eventually these reactions became nearly independent of O_2 levels and this means of buffering O_2 levels was lost [*cf.* 5]. In effect, the easily oxidized sinks were titrated.

High methane would also explain Archean greenhouse warming when the sun was 20-30% less luminous [3]. Paleosols indicate $pCO_2 < 0.04$ bar at 2.75 Ga [6], insufficient by an order of magnitude to prevent Earth from freezing. In a methane-rich Archean atmosphere, we deduce pCO_2 to have been low (<0.1 bar) via negative feedback. CO_2 augmentation to methane-dominated greenhouse warming would have led to rapid removal of CO_2 via silicate weathering. During the oxic transition, methane would oxidize with significant loss of greenhouse warming. This is consistent with the global glaciation at 2.4-2.2 Ga that coincides with the rise of O_2 according to Huronian paleosols [3,7].

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Constraints on Early Mantle Redox State

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Recent work by Delano (in press, 2000) and Canil (1997) indicates that Earth's mantle was at or near its present oxidation state, the fayalite-magnetite-quartz (FMQ) buffer, since the Archean and possibly as far back as 3.9 Ga. A FQM mantle with an oxygen fugacity of 10^{-7} to 10^{-8} would produce volcanic gases dominated by water (H_2O) and carbon dioxide (CO_2), with trace amounts (~1-3%) reduced gases such as hydrogen (H_2) and carbon monoxide (CO). This presents problems for origin of life theories that rely on reduced gases and lightning to produce the first prebiotic molecules for life (e.g., Miller, 1953). A mantle at or near the iron-wustite (IW) buffer with oxygen fugacities around 10^{-12} *would* volcanically outgas more H_2, CO, and CH_4 (methane), producing a more reduced atmosphere that would have been more conducive to the formation of life *in situ*. In addition, it has been shown that the Moon has a redox state near IW (Delano, 2000). If the Moon was formed from a Mars-sized impactor that hit Earth and retained Earth-mantle material, this is an indicator that the early mantle *was* more reduced, possibly near IW. In this work, we investigate this possibility, suggested earlier by Kasting, et al. (1993), and discuss how Earth's mantle may have evolved in redox state from IW to FMQ through hydration, followed by outgassing of H_2.

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How Does *C. elegans* Respond to Altered Gravity?

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All organisms on Earth have evolved at unit gravity (1xG), and thus are probably adapted to function optimally at 1xG. However, with the advent of space exploration, it has been shown that organisms are capable of surviving at much less than 1xG, as well as at greater than 1xG. Organisms subjected to increased G levels exhibit alterations in physiological processes that compensate for novel environmental stresses, such as increased weight and density-driven sedimentation. These physiological adaptations illustrate the plasticity of organisms when presented with environmental conditions in which they could not possibly have evolved. Investigating the mechanism(s) behind these adaptations may uncover biological pathways that have not previously been identified. An easily-cultured and well-studied organism, such as *C. elegans*, would be a desirable model system for these studies. However, the effects of increased G are dependent on size, and the lower size limit for responding to increased G has not been established. *C. elegans* are routinely subjected to transient accelerations of several thousand times G, during common laboratory procedures requiring centrifugation, and show no obvious ill effects. Even a transient exposure to 100,000xG did not result in permanent damage (L. Avery, personal communication). In order to establish whether *C. elegans* exhibit altered physiological processes as a result of several days exposure to low levels of increased G (2-10xG), we have developed a centrifugation protocol using axenic liquid cultures of *C. elegans* and the 1-foot diameter centrifuge at NASA-Ames Research Center. During exposure to 5 or 10x G for 4 days*, C. elegans* cultures exhibited retarded growth relative to stationary control cultures. Immediately after being removed from the centrifuge, *C. elegans* were immobile and did not display a 'tap-response' to brief mechanical stimulation. Mobility and the tap-response were regained by two hours after the return to unit gravity, and the behavioral responses and growth of cultures were subsequently indistinguishable from controls. Growth at 10xG for 4 days produced alterations in steady-state mRNA levels, as evaluated by whole-genome microarray analysis on mRNA from centrifuged *C. elegans* compared to parallel cultures of stationary controls. Fewer than 100 genes out of 19,600 were upregulated or downregulated to a statistically significant degree. Only a few stress-response genes were upregulated, indicating that the centrifuged cultures were under mild stress. Most of the genes are of unknown function, but a number of them show similarity to proteins possibly involved in signal transduction, including G-protein receptor subunits and transcription factors. These data demonstrate that *C. elegans* do respond to increases in G level. A series of time-course and G-level exposures are being performed, to establish how the genes that we have identified may be involved in the responses of *C. elegans* to increased gravity.

Late Archean Biomarkers in Carbonates from the Hamersley Basin, Pilbara Craton, Western Australia

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Little is known about the extent of microbial metabolic diversity in the Archean. Detection of indigenous molecular fossils and the assessment of the isotopic expression of carbon and sulfur in sediment give us the potential to reach an improved understanding of microbial evolution and its role in biogeochemical cycles. Here we report the distribution of hydrocarbon biomarkers detected in diamond drill core carbonate samples of a late Archean (2.8-2.5Ga) rock succession from the Hamersley Basin on the Pilbara Craton, Western Australia. Our data suggest the preservation of molecular fossils from metabolically diverse ancient ecosystems of carbonate environments.

Bitumen from rocks of the WRL1, RHDH2a, and SV1 drill cores, comprising the Mt. Jope and Marra Mamba Supersequences (i.e. Fortescue and Hamersley Groups), were extracted under tightly controlled and monitored laboratory conditions. This rock suite represents various depositional environments including, the shallow and deep-water facies of a carbonate platform and stromatolitic carbonates of either a lacustrine or restricted marine basin.

The 2.7-billion year period between sedimentation and biomarker analysis means extra-ordinary measures must be taken to make certain an indigenous and syngenetic relationship exists with the host rock. Biomarker maturity parameters, such as TA(I)/TA(I+II), $\alpha\alpha\alpha$ C_{27} sterane 20S/(20S+20R), C_{27} sterane $\alpha\beta\beta/(\alpha\beta\beta+\alpha\alpha\alpha)$, and C_{27} trisnorhopane Ts/(Ts+Tm), indicate thermal maturity well past the conventional "oil window" in accord with regional geology. Plant biomarkers were not detected. Variations in biomarker parameters within individual lithofacies and between closely spaced samples suggest petroleum contamination is not present.

Biomarkers identified in carbonates include *n*-alkanes, monomethylalkanes and a range of steroids and hopanes. These observations are similar to those by Brocks et al.

(*Science,* 285, 1033-1036, 1999) on shales from the same rock succession. The presence of 2α-methylhopane in all samples supports the antiquity and widespread presence of oxygenic photosynthesizers (i.e. cyanobacteria). Diverse saturated steranes and aromatic steroids indicate the sterol biosynthetic pathway, most characteristic of the Eukarya domain, had evolved by 2.72 Ga. Biomarkers never before detected in Archean samples, include several homologous series of dimethylalkanes and, most significantly, the trace presence of 3β-methylhopane. Methylotrophic and acetogenic bacteria are known precursors of 3β-methylhopane, thus its presence imparts potential evidence for aerobic recycling of methane consistent with Hayes' hypothesis for the origin of strong ^{13}C depletion in late Archean kerogens.

Dimethylalkanes were detected in both shallow and deep-water carbonates but, most strikingly, only in laminated lithologies. In particular, one or more series were found in a "rollup" laminated limestone of the Wittenoom Formation (WRL1), a stromatolitic laminated carbonate of the Tumbiana Formation (SV1), and finely laminated dolomitic black shales of the Lewin Shale (7 samples from RHDH2a). Other researchers have identified similar dimethylalkanes (and monomethylalkanes) in modern and lithified mats, thus the presence of these compounds in Archean rocks supports a microbial mat interpretation of laminae and a syngenetic relationship with the host rock. Microbial mats are generally composed of a tight-knit mixed community of organisms living in microenvironments that regulate oxygen availability. In composition, a plethora of organisms have been associated with mats, including aerobic and anaerobic photosynthesizers, chemoautrophs, and heterotrophs, consistent with the diverse metabolisms suggested by the distribution of biomarkers in these rocks.

A clearly distinguishing feature of carbonate extracts from all three cores is the bimodal *n*-alkane distribution (maxima at nC_{17} and nC_{23}) observed in 8 of the 11 carbonate samples analyzed. Shale *n*-alkane distributions observed by Brocks et al. and in 19 samples of this study have a unimodal distribution with maxima at $\sim nC_{17}$. Although this pattern could be an artifact imprinted by host rock lithology associated with bitumen redistribution, it could also be the result of an additional biological organic source associated with carbonate deposition, and, if so, would provide an additional argument for indigenous molecular signatures.

Carbon-isotope analyses are being conducted in order to assess microbial carbon cycling in more detail and to address the origin of -40 to -60‰ δ^{13}C kerogens measured for samples from these rock units.

Biotic Recoveries from Mass Extinctions: Insights into Evolutionary Innovation

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Biotic recoveries following mass extinctions involve the rebuilding of ecological communities following massive perturbations, and often display significant biological innovations. Consequently they serve as important systems to examine the interplay between genetics/development, environment and ecology in evolutionary innovation. Significant apparent evolutionary innovations include the spread of angiosperms and placental mammals following the end-Cretaceous mass extinction, ammonoids after the end-Devonian, end-Permian and end-Triassic mass extinctions, echinoids following the end-Permian and end-Triassic mass extinctions and caenogastropods after the end-Permian mass extinction. Yet the spread of placental mammals and angiosperms does not mark the origin of either clade, and may not mark the origin of any significant subclades. In each case, molecular evidence suggests the clades may pre-date the extinction, with their diversification being an ecological response to the mass extinction. Ammonoids considerably predate each biotic crisis they suffered, and although some significant innovations do occur during their history, the pattern of biotic overturn appears to also follow an ecological model. Caenogastropods and echinoids, in contrast, exhibit patterns of morphological innovation more closely linked to the post-extinction biotic recovery. Thus there is no close correlation between developmental and morphological inventions (to use an analogy from the study of techonological innovation) and their ecological spread as innovations.

This seems to suggest that ecology is the most significant control in innovation, a view incorporated in many models. Most models of post-extinction recovery are modifications of a simple logistic growth model, implicitly assuming the existence of ecological niches independent of any species occupying the niche (an assumption most ecologists realize is invalid). Applied to biotic recoveries, such models include an immediate biotic response following the end of the extinction, and logistic growth to a new equilibrium. The validity of several of these assumptions is questionable. New models of biotic recovery in which species create their own niches, and involving positive feedback show promise in more closely matching empirical studies of some post-extinction biotic recoveries.

The study of biotic recoveries serves as an important testing ground for the study of evolutionary innovations in general. Most discussions of evolutionary innovation fall into two classes: key innovation models and open ecospace models. Both involve access to previously unavailable resources. In the first, the appearance of a new feature drives acquisition. In the second, an underutilized resource is made available, perhaps through occupation of a new offshore island, or following mass extinction. Both models follow the standard view of: “genetic/development proposes, ecology disposes.” The constructionist view of biotic recoveries suggests a need to modify this approach to evolutionary innovations, with development and ecology constructing aspects of the environment and thus the ecological niches. Such a perspective has implications for major evolutionary transitions.

Genes Involved in Photosymbiotic Interactions

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We are interested in understanding how photosymbiotic relationships are controlled through the genetic communication of two cells, and perhaps, how this may relate to the evolution of organelles. The presence of an algal symbiont represents the acquisition of novel functions by the host cell. There is an increasing body of evidence for the eukaryotic origin of chloroplasts in cryptophytes, chlorarachniophytes, euglenophytes, heterokonts, haptophytes and dinoflagellates. In these different hosts, the endosymbiotic algae are in various states of genetic and physical reduction. In all cases it is impossible to remove the alga from its host and culture it independently.

Planktonic foraminifera, radiolaria and acantharia are ameboid protists that occur in the pelagic environment of the World's oceans. Many of the species in this group harbor algal symbionts, particularly dinoflagellates. These symbiotic relationships have not evolved to the point where the symbiont is dependent upon the host, so it is possible to separate the two and study the changes in algal gene expression that occur when the free-living organism becomes a symbiont. The sarcodine symbioses will also allow the comparison of symbiotically regulated genes in taxonomically distinct algae, which can lead to the identification of the common genetic elements involved in the associations, as well as what makes each one specific.

In order to reach our overall goal of determining the genetic mechanisms behind algal symbioses and, potentially, organelle evolution, we have begun comparing the mRNA expressed in symbiotic and free-living dinoflagellate symbionts from the radiolarian *Thalassicolla nucleata*. We have cultures of the dinoflagellate (*Scrippsiella velellae*) in our laboratory, which serve as a source of free-living RNA. To obtain RNA from the symbiotic state of *S. velellae*, we collected intact symbioses and dissected the symbionts away from the host. RNA was isolated from cultures and dissected symbionts using Ambion's RNAqueous Kit. The two samples of RNA were processed further by suppression subtractive hybridization. Messenger RNA is converted to cDNA, and hybridization of cDNA tester (symbiotic cDNA) with cDNA driver (free-living cDNA) results in a population of cDNAs enriched for those of the tester (symbiotic). The differentially expressed cDNAs are amplified, and the PCR products are cloned and screened for confirmation of differential expression. This process enriches/selects for genes that are differentially regulated and is available in a kit format from Clontech.

We have completed a subtractive selection for *S. velellae*, and have recovered over 20 differentially expressed clones. Sequence analysis of these clones has indicated that 11 are essentially identical (C4). Although their Blast searches recover unidentified human chromosomal DNA as the most similar sequences, there is actually not a significant match. We are continuing to analyze the clones that we have already obtained, in particular the C4 clones, by performing genomic blots to confirm that the sequences are present in the algal genome, and will then pursue recovery of the whole gene through cloning.

Modeling the Evolution of Food Webs Under Biogeochemical Constraints

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Biological stoichiometry is the study of the balance of energy and multiple chemical elements (e.g., C, N, P) in living systems. Over the last decade, biologists have shown that stoichiometric relationships—in particular disparities in the relative availability of chemical elements in autotrophic resource species in comparison with the relative demand for those elements in the body tissues of consumer species—can affect the behavior, population stability, and evolution of species, the structure and dynamics of ecological communities, and biogeochemical cycling in food webs. However, such stoichiometric principles are not present in traditional models for exploring the complexity of ecological systems. Under the auspices of NASA's Astrobiology program, we are developing a new conceptual framework for exploring species evolution and the development of speciose ecological communities under stoichiometric constraints. Specifically, we aim to understand how species evolution and community development depend upon ecosystem-level factors (e.g., chemical nutrients, photosynthetically active radiation) that restrict species' life strategies. In our model, evolution and community assembly occur within a model ecosystem, where the stoichiometry of resources constrains both the exploration of adaptive landscapes and food-web complexity. Eventually, we hope that these theoretical efforts will provide insight into the development of living systems on Earth (and elsewhere?) by connecting evolving, interacting lineages to the chemical properties of living systems. In particular, stoichiometric considerations may prove relevant to diversification-extinction dynamics during key periods in earth's history such as the Precambrian-Cambrian boundary, the colonization of terrestrial habitats, and mass extinction events.

A Genomic Timescale for the Origin of Eukaryotes

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Genomic analyses have shown that horizontal gene transfer occurred during the origin of eukaryotes following symbiosis. However, the number of symbiotic events and their relationship with changes in Earth's environment are unclear. Our protein sequence analyses support two symbiotic events: one pre-mitochondrial and a later mitochondrial event. Molecular time estimates of these events, and of the origin of cyanobacteria, suggest that the innovation of oxygenic photosynthesis had a relatively rapid impact on the environment and evolution of eukaryotes.

Microbial Mats as Self-Regulating Systems

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We have adopted a numerical model of microbial mat biogeochemistry (*de Wit et al., 1995, FEMS Microb. Ecol. 17:117-136*) to investigate the properties of this important ecosystem. Specifically we are interested in the ability of the microbial mat ecosystem to regulate nutrient and light availability, and to damp the effects of external and internal stresses. The persistence of these ecosystems for billions of years on Earth, and the likelihood of their existence elsewhere, depends in part on their resilience to environmental perturbation.

A number of scenarios are being investigated, including the response of mats to diurnal and seasonal variations in light availability, and changes in specified heterotrophic activity (aerobic respiration and anaerobic sulfate reduction). Of relevance to studies of the Archean Earth are simulations where the mat is subjected to reduced (and elevated) surface O_2 concentrations and UV light intensity. Various stabilizing feedback loops exist; their sensitivity to various parameters is investigated quantitatively.

Using Homolog Groups to Create a Whole-Genomic Tree of Free-Living Organisms: An Update

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Evolutionary relationships and significant evolutionary events can be studied using whole genome sequences in many different ways, including the building of genomic trees using methods based on the presence and absence of genes in each genome [1]. Several different methods for the generation of trees using gene content hasve been proposed [2, 3, 4, etc.]. These different processes for building genome trees can be divided into two broad categories: those based on the presence and absence of suspected ortholog pairs or the "Ortholog method" [3] and those based on the presence and absence of gene families or "the Homolog method" [4].

Each of these distinct methods of genome tree building has advantages and disadvantages. The ortholog method is quite effective at recovering an overall average of the different phylogenetic histories for the genes in the genomes studied. Further, because "evolutionary distance" in this method is based on the proportion of orthologs shared between two genomes divided by the size of the smallest genome of the two, the method is not prone to artifacts caused by differing genome size. However, the ortholog method can in principle be greatly influenced by lateral gene transfer as recently transferred genes will appear as orthologs and by the loss of shared genes or the duplication of unshared genes [1]. In contrast, the homolog method can be affected by greatly reduced genomes, but is resistant to influences of most lateral gene transfer because only the transfer of novel gene families can influence the observed distribution of characters in the tree-building process. Furthermore, expansions and reductions of gene families through duplications or limited gene loss has no influence on the tree-building process because all gene families are treated equally, regardless of their size.

Since the publication presenting the homolog method of genome tree construction [2], several more complete genome sequences have become available with which to test and evaluate this method of genomic tree construction, as well as, with which to continue to explore genome evolution in a greater diversity of species. The data set used in this analysis includes genome sequences from 27 species, including 15 free-living taxa. Here, using these genome sequences, we present tree construction using all 27 taxa, tree construction using only the 15 free-living taxa, an analysis of the influence of certain laterally transferred genes, and lastly an evaluation showing that the method is often able to robustly recover the genome tree topology when only partial genomes are available.

The genomic tree of the 15 free-living taxa includes six different bacterial orders, six different archaeal orders, and two different eukaryotic kingdoms. The results are remarkably similar to results obtained by analysis of rRNA. Inclusion of the other 12 (non-free-living) genomes resulted in a tree only broadly similar to that suggested by the rRNA, with differences due to artifacts caused by the very small genome size of many of these species. Very small genomes, such as those of the two *Mycoplasma* genomes included, fall to the base of the Bacterial domain, a result expected due to the substantial gene loss inherent to these lineages. Finally, "partial genomes" were generated randomly from the complete genomes used in order to test our ability to recover the tree generated by the whole genome sequences when only partial data are available. The results indicated that partial genomes, when sampled randomly, could robustly recover the tree generated by the whole genome sequences.

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Towards an Understanding of the Late Archean Sulfur Cycle

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The isotope records of sedimentary sulfides and sulfates have been used to interpret the biogeochemical cycling of sulfur and constrain global redox levels. The sulfur isotopic composition of sedimentary pyrite is influenced by a significant kinetic isotope effect that occurs during bacterial sulfate reduction as obligate anaerobes preferentially dissimilate the lighter ^{32}S in the production of H_2S and pyrite. A negative $\delta^{34}S_{pyrite}$ excursion occurs at ca. 2.3 Ga. The sulfur isotopic composition of sedimentary sulfates and sulfides deposited before 2.3 Ga cluster around the mantle value of 0 ± 5‰. One interpretation of the minimal fractionation between sulfates and sulfides ($\Delta^{34}S_{sulfate-pyrite}$) is that pyrite formed from magmatic sulfide rather than biogenically produced sulfide. The assumption is that the pre-2.3 Ga ocean was sulfate-poor, and that with essentially no dissolved sulfate there would be no bacterial sulfate reduction and, therefore, minimal isotopic fractionation. A contrasting interpretation is that the minimal fractionation is a result of very rapid rates of sulfate reduction in a relatively sulfate-rich ocean. Experimental evidence suggests that increased rates of sulfate reduction diminish the discriminating effects of bacterial isotope fractionation.

At the very heart of the issue is whether Archean oceans contained appreciable sulfate concentrations. Sulfate should have reached significant concentrations, at least in surface waters, as soon as oxygen rose to levels within the atmosphere sufficient to promote non-biologically mediated sulfide oxidation in soils, etc. Unfortunately, the timing of the rise in atmospheric oxygen remains equivocal. However, trace amounts of sulfate are incorporated into marine carbonates in proportion to the sulfate concentration in the original solution and the rate of crystal growth. The present study employs a method for extracting trace sulfate from carbonates in order to document oceanic sulfate availability and secular variations in the sulfur isotopic composition of late Archean oceanic sulfate.

Preliminary analyses indicate that Late Archean (~2.6 Ga) carbonates from Western Australia (Wittenoom and Carawine Dolomites of the Hamersley Group) contain 0 to ~50 ppm sulfate with $\delta^{34}S_{sulfate}$ values ranging from ~2 to 22‰. For comparison, Neoproterozoic marine carbonates contain 3 to 295 ppm sulfate with $\delta^{34}S_{sulfate}$ values ranging from ~10 to 40‰ and Holocene marine carbonates (microfossils and bulk carbonate) contain 200 to 750 ppm sulfate. The fact that Archean carbonates contain any trace sulfate suggests that oceanic surface waters in the Late Archean, at least periodically, contained some sulfate. Additionally, the relatively enriched $\delta^{34}S_{sulfate}$ values suggest that sulfate-reducing bacteria were active during the late Archean. There is some indication that $\delta^{34}S_{pyrite}$ and $\delta^{34}S_{sulfate}$ values for the Carawine Dolomite are enriched in ^{34}S and that sulfate concentrations are higher compared to the Wittenoom Dolomite, the deeper water equivalent. These data suggest that marine surface waters may have contained more sulfate than deeper waters and could support a larger bacterial sulfate reducing community.

The Sulfur Isotopic Composition of Neoproterozoic Seawater Sulfate: Implications for a Snowball Earth

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Biogeochemical processes impose significant and predictable isotopic fractionations on sulfur species as they are cycled between oxidized (i.e., sulfate) and reduced (i.e., sulfide) forms. Consequently, the isotope records of sedimentary sulfides and sulfates are sensitive indicators of environmental change. Sedimentary pyrite most commonly forms as a result of bacterial sulfate reduction and its common occurrence in sedimentary rocks has enabled researchers to partially reconstruct the evolution of $\delta^{34}S_{pyrite}$ through the Neoproterozoic. In order to assess the true extent of fractionation and interpret variations in $\delta^{34}S_{pyrite}$, the contemporaneous $\delta^{34}S_{sulfate}$ must be evaluated. Previous secular $\delta^{34}S_{sulfate}$ trends for the Neoproterozoic have been developed using rarely occurring evaporitic gypsum and barite deposits that provide only a low temporal resolution of sulfate evolution. However, trace amounts of sulfate incorporated into marine carbonates can be analyzed for its S-isotopic composition as an indicator of seawater $\delta^{34}S$ history. Carbonates are significantly more abundant and relatively continuously deposited in the Neoproterozoic than sedimentary sulfate deposits and therefore can provide a relatively continuous marine sulfate sulfur isotopic signal that may be utilized to assess environmental change.

The Neoproterozoic (1000–545 Ma) is of particular interest because it has been characterized as a time of dramatic environmental and biological change. At least two distinct global glaciations may have been responsible for covering most, if not all, of the world's oceans with sea ice (Harland, 1964; Kirschvink, 1992; Hoffman et al., 1998). The sensitivity of $\delta^{34}S_{sulfate}$ to biogeochemical change makes it a candidate to evaluate aspects of the Snowball Earth hypothesis, particularly whether or not the oceans were effectively isolated from air/sea exchange and riverine runoff as the result of long-term global sea-ice cover (Hoffman et al., 1998). The present study utilizes trace sulfate extracted from Neoproterozoic carbonates (Namibia and South Australia) to track the sulfur isotopic composition of oceanic sulfate and to assess variations in the sulfur cycle that may have accompanied the 'Snowball Earth' events. The nearly pure carbonates in Namibia and South Australia contain 3 to 295 ppm sulfate.

The $\delta^{34}S_{sulfate}$ values not associated with the two glacial intervals (~740 and 600 Ma) in Namibia exhibit significant shifts over tens of millions of years, but vary only between ~15 and 25‰ (CDT). Dramatic positive excursions, reaching 40‰, appear stratigraphically above the lower (Chuos Fm) and upper (Ghaub Fm) glacial intervals in what have been termed 'cap carbonates.'

We hypothesize that the large positive $\delta^{34}{}_{sulfate}$ excursions found in Namibian Neoproterozoic cap carbonates and the relationship between $\delta^{34}S_{sulfate}$ and $\delta^{13}C_{carbonate}$ are consistent with the "Snowball Earth" events outlined by Hoffman et al. (1998), although, there are other mechanisms that may facilitate these large shifts in $\delta^{34}{}_{sulfate}$. If the Earth's oceans were covered with ice for millions of years, the source of relatively depleted $\delta^{34}S$ from the continents via pyrite weathering would disappear. If sulfate-reducing bacteria continued to function and preferentially dissimilate ^{32}S, the isolated oceanic sulfate pool would be driven to more enriched $\delta^{34}S$ values and the concentration decreased. During deglaciation, ocean stratification probably broke down and deepwater overturn occurred. As a result, the trace sulfate associated with post-glacial carbonates was lower and enriched in ^{34}S. As the delivery of sulfur via riverine inputs was reestablished, the $\delta^{34}S_{sulfate}$ gradually returned to more typical values of 20‰ over perhaps tens of millions of years. Neoproterozoic $\delta^{34}S_{pyrite}$ records from western Canada and Australia (Amadeus Basin) parallel our $\delta^{34}S_{sulfate}$ results from Namibia. The generally small $\Delta^{34}S_{sulfate\text{-}pyrite}$ values suggest that reduction of sulfate by bacteria was complete at these sites. Additionally, sulfate concentrations in Neoproterozoic carbonates, the volatile nature of the $\delta^{34}S_{sulfate}$ curve and the apparently full reduction of sulfate (as implied from $\delta^{34}S_{pyrite}$ data) suggest that Neoproterozoic oceanic sulfate concentrations were lower than modern values.

By contrast, the 'cap carbonates' from South Australia (Nuccaleena Fm) are characterized by $\delta^{34}S_{sulfate}$ values that vary only between ~10 and 20‰ and do not exhibit the dramatic positive excursions developed in the Namibian samples as well as the $\delta^{34}S_{pyrite}$ excursions recorded in western Canada and the Amadeus Basin. One possibility is that the Nuccaleena Formation was not deposited at the same time as the Namibian, western Canada and Amadeus Basin carbonates and, for example, did not begin accumulating until after the delivery of sulfur via riverine inputs was reestablished. A second possibility is that the Nuccaleena Fm is recording sulfate values of a completely different water mass (i.e., separate ocean basin). These possibilities can be evaluated with other data, such as carbon isotope signatures, etc.

Photochemical Modeling of Mass-Independent Sulfur Isotope Fractionation in Low-O_2 Atmospheres

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Farquhar et al. [1] have presented evidence for mass-independent sulfur isotope fractionation in rocks of Archean and early Proterozoic age. Such fractionation is not observed in younger rocks. The most likely mechanism for producing mass-independent fractionation involves photochemical reactions initiated by photolysis of either SO_2 or H_2S. Farquhar et al. suggested that atmospheric O_2 and O_3 levels must have been low during the Archean to allow these sulfur gases to be photolyzed.

We agree with Farquhar et al. regarding the general requirement for low O_2 in the Archean. However, the actual upper limit on O_2 should be set, not by photolysis considerations, but by the requirement that sulfur species can leave the atmosphere in different chemical forms. The mass-independent fractionation of sulfur isotopes is probably created by a photochemical network similar to that shown schematically in Fig. 1. Sulfur is emitted from volcanos in the form of either SO_2 or H_2S. Photochemical

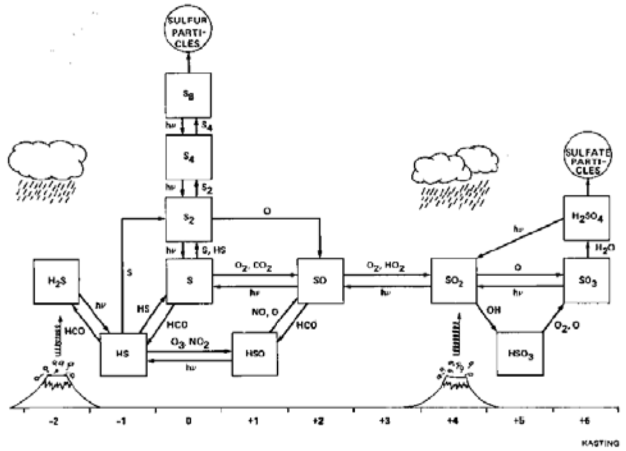

Fig. 1 Diagram illustrating sulfur photochemistry in a low-O_2 Archean atmosphere. The horizontal axis shows the oxidation state of sulfur. From ref. [2].

reactions cause the H_2S to be oxidized and the SO_2 to be either oxidized or reduced. Sulfur can then leave the atmosphere in at least 4 different chemical forms (H_2S, S_8, SO_2, and H_2SO_4). Some of these gases (S_8 and H_2SO_4) form aerosol particles that can sediment out. All gases except for S_8 are soluble to some extent in water. Thus, mass-independent fractionations created by photolysis can be preserved in different chemical species, each of which will have a different potential for incorporation into sediments. By contrast, in today's O_2-rich atmosphere, virtually all sulfur gases undergo oxidation and exit the atmosphere as H_2SO_4. Thus, even if sulfur isotopes were to be mass-independently fractionated by photochemical processes, the isotopes would be re-homogenized when the sulfur entered the ocean. This, by itself, is a strong argument that the Archean atmosphere must have had extremely low O_2 levels.

We are attempting to quantify this argument by developing a numerical model of sulfur isotope fractionation. This model will build on previous paleoatmospheric models [3,4]. Preliminary results from this modeling should be available by the time of the conference.

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Proterozoic CO_2 Levels Estimated from *in-situ* Carbon-Isotopic Compositions of Organic-Walled Microfossils

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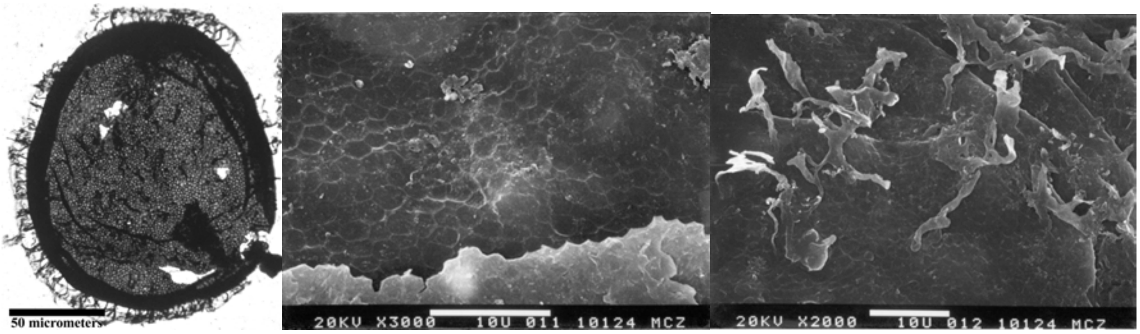
Ion microprobe analyses of robust, organic-walled microfossils – macerated from billion-year-old shales – indicate magnitudes of carbon-isotopic fractionation consistent with elevated levels of CO_2 in the ancient atmosphere, assuming Calvin cycle metabolism.

The acritarchs (considered to be an encystment phase of the protist life cycle) used in this study consisted of a single species (*Shuiyousphaeridium macroreticulatum*) of 150+ micron diameter microfossils from the Ruyang Group in Shanxi Province. Granites that intrude overlying sediments are dated at 999 Ma. Microscopic and SEM investigations reveal a complex of interlocking polygonal plates on the acritarch surface as well as an irregular distribution of 5-10 micron long tubular spines.



Their size and complex morphology suggest that these acritarchs were most likely photosynthetic eukaryotes and, by inference, used the Calvin cycle for carbon fixation. Although similarly sized sulfur-oxidizing bacteria have been found in some modern environments, these are morphologically simple and are far less likely to be preserved in the rock record due to the absence of an encystment stage with robust walls. Samples were imaged down to 100 nanometers to evaluate potential surface contamination of the acritarch surface by bacteria with the University of Maryland field emission SEM.

The Carnegie Institution 6f Cameca ion microprobe was used to measure the carbon-isotopic composition of these organic-walled fossils. The primary Cs^+ beam was focused down to a 20-25 micrometer spot, which allowed for multiple analyses of the same individual. Over $1e^6$ counts per second were recorded on the major $^{12}C^+$ secondary beam during analyses of both the standard Mao Diamond and microfossils. The carbon-12 hydride peak was clearly resolved from those of $^{13}C^+$ ions and did not interfere in the isotopic measurement. Multiple analyses of the standard over three consecutive days revealed better than 0.3‰ external reproducibility of the analytical technique (95% confidence interval); variance between multiple analyses ($n = 5$) of the same microfossil in different spots was about 1.5‰.

The carbon-isotopic composition of all individuals ranged between –33.5 to –36.3‰ vs. PDB, which on average records the theoretical maximum fractionation between reactants and products of the Calvin cycle. Chemostat studies of modern marine eukaryotic algae show that maximal carbon-isotope fractionation in the Calvin cycle is attained at elevated pCO_2 (8-10x pre-industrial levels). Our results are consistent with predictions of higher greenhouse gas concentrations in the early atmosphere, as well as higher alkalinity and dissolved inorganic carbon concentrations inferred from the predominance of seafloor carbonate cements in the Precambrian stratigraphic record.

NAI Evolutionary Genomics Focus Group Plans for Investigating the Metazoan Radiation (Cambrian Explosion) in the Context of the Snowball Earth

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The goal of this research initiative is to examine the influence of geologic and planetary-scale climatic events during the Neoproterozoic, 1000-545 million years (Ma) ago, on the early evolution of metazoans (animals). The first undisputed evidence of animals in the fossil record is in the latest Neoproterozoic (~570 Ma) and most major groups appear shortly thereafter in the "Cambrian Explosion" which accelerated dramatically about 545 Ma ago. However, virtually all molecular estimates of divergence times made over the last three decades have indicated a much earlier period of animal evolution which is unrecorded in the fossil record and perhaps began as early as 1,600 Ma ago. It has been suggested that early animals were small and soft-bodied, which would explain their absence from the fossil record. If true, animals would have had to survive planetary-scale climatic events ("Snowball Earth" events) of the Neoproterozoic that occurred between about 750 and 600 Ma ago. The environmental conditions that existed during Snowball Earth periods have been debated, but even under the mildest of scenarios, they would have been challenging for any complex organism.

Understanding how Earth's life was affected by these major environmental perturbations will help us understand how life might have evolved and adapted elsewhere in the Solar System and beyond. However, the single greatest limit to progress at present is our incomplete knowledge of the relationships and timing of animal evolution. Therefore, we plan to obtain DNA sequences of large numbers of genes from representatives of most of the major groups of animals. A robust evolutionary framework that is well tied to the fossil record will permit hypotheses about rates, relationships, and divergence times to be tested and, at the same time, guide future studies on all aspects of animal evolution, including development, gene function, and adaptation.

Constraining Sulfate and Oxygen Availability in the Mesoproterozoic Ocean

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Unambiguous constraints on seawater compositions are among the more vexing challenges in Precambrian biogeochemistry. We discuss here two methods, one proven and one in the developmental phase, that speak to two of the more important dissolved species in the Precambrian ocean: oxygen and sulfate. Both methods, Fe/Al (Mo/Al) ratios in shales and trace sulfate in carbonates, are relatively simple in theory and in application but have strength in the broad availability of suitable samples and in the potential for preserving primary signals over complex burial histories.

Canfield (1998, *Nature*) proposed that the ocean was anoxic until the Neoproterozoic when a global-scale event triggered the onset of oxygenation throughout the water column. Despite this assertion, constraints on the depositional redox chemistry of the Proterozoic ocean remain elusive. Traditional proxies for water-column oxygen concentrations commonly fail when applied to Proterozoic sediments, including the simple use of presence or absence of lamination to indicate benthic macrofaunal activity, as is widely applied in the Phanerozoic. As a result, models for Precambrian oxygenation commonly rely on terrestrial records (e.g., paleosol redox relationships) or indirect marine geochemical indicators, such as sulfate availability as inferred from the distribution of gypsum through time. Beyond inherent ambiguities, these methods are weakest when used to address local-scale patterns in marine oxygen availability. Given the lack of Proterozoic bioturbating organisms and the failure of the other traditional approaches, laminated Proterozoic black shales are less clearly products of anoxic deposition than their Phanerozoic equivalents.

Recent work in modern anoxic settings (e.g., Black Sea and Cariaco Basin) has shown that Fe/Al ratios in fine-grained siliciclastic sediments are controlled by the presence or absence of hydrogen sulfide in the water column. In anoxic-sulfidic environments, the Fe/Al ratio is elevated relative to oxic sediments due to the scavenging of dissolved iron during water-column (syngenetic) pyrite formation. Delivery of this scavenged Fe is decoupled from the local terrigenous influx and therefore can drive up the Fe/Al ratio in anoxic basins during periods of low sediment accumulation. The basic hypothesis asserts that Fe/Al ratios in Proterozoic deep-water black shales will record the availability of oxygen during deposition and in doing so test basic models for the distribution of anoxia in the Proterozoic ocean. More specifically than addressing the absence of oxygen in the ocean, this method is intrinsically linked to the presence of hydrogen sulfide in the water column. Unfortunately, few data exist for coeval, shallow-shelf red shales of known oxic origin. These red shales, the focus of our ongoing study, provide the essential Fe/Al baseline for the continental influx, against which anoxic Fe enrichment and corresponding distributions of Proterozoic marine anoxia can be measured. It is our assertion that these red shales represent the oxic shallow waters of an ocean that may have otherwise been largely anoxic and sulfidic at depth. In this presentation, we will discuss the theory behind this method, results from modern and ancient Phanerozoic settings (e.g., Devonian and Pennsylvanian) and our preliminary results from Precambrian shales. Throughout, we will emphasize the importance of comparing data from temporally and spatially proximal red shales to the results for laminated black shales of uncertain paleoredox origin. We will also highlight analogous relationships for Mo/Al, wherein Mo is effectively scavenged (enriched) in the sulfidic water column so that, as for iron, the magnitude of enrichment relative to oxic crustal end members reflects the presence of sulfide in the water column in combination with the extent of dilution from the detrital influx.

The balance between oxidation and reduction of sulfur at the earth's surface is intimately linked to the availability of oxygen in the coupled ocean-atmosphere system. This balance manifests as broad temporal shifts in the sulfur isotope composition of seawater sulfate and is well recorded in gypsum deposits throughout the Phanerozoic. In the near absence of gypsum in pre-Phanerozoic sediments, trace amounts of sulfate trapped within abiotic limestones and dolostones of Precambrian successions can be used to constrain the amount and isotopic composition of sulfate in ancient seawater. Our results to date for this carbonate-associated sulfate (CAS) in Mesoproterozoic samples show up to 10 permil sulfur isotope variation over relatively thin stratigraphic intervals. At one locality, this isotopic variability in CAS is corroborated by trends within coexisting gypsum. When compared to the temporally broader shifts of the Phanerozoic, this rapid Mesoproterozoic sulfur isotope response suggests an appreciably smaller oceanic sulfate reservoir. Such limitations in sulfate availability, presumably constrained by lower levels of ocean-atmosphere oxygen in the Proterozoic, may also be reflected in the global scarcity of gypsum prior to approximately 1.3 Ga and the abundance of isotopically heavy bacteriogenic iron sulfides. Model results will be presented linking inferred seawater sulfate concentrations to rates and magnitudes of sulfate-S isotope variability in the global ocean. CAS results will be discussed from the ~1.4 Ga Belt Supergroup of the northwestern United States, the ~1.2 Ga Apache Group of central Arizona, the ~1.2 Ga Bylot Group and ~1.3 Ga Dismal Lakes Group of arctic Canada, as well as the ~1.7 Ga McNamara Group of northwestern Queensland. Ultimately, our ability to integrate Fe-Mo-CAS records will allow construction of a comprehensive model for Mesoproterozic oceanic oxygen availability on both local and global scales.

Geology and Age of Supracrustal Rocks, Akilia Island, Greenland: New Evidence for a >3.83 Ga Origin of Life

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Supracrustal rocks on and near Akilia Island, southwest Greenland, have been proposed to contain both the oldest marine sediments in the geologic record [1], and the oldest evidence for active biological processes [2]. These interpretations rely on three strongly contested geologic observations, including (1) the nature of crosscutting relations that constrain the age of sediments that host biosignatures; (2) the sedimentary origin of some lithologies; and (3) the interpretation of zircon ages [3]. Despite heated debate about this locality and its geologic significance, the exposure has never been mapped or sampled on a scale appropriate to address the contested issues. In July 2000, we mapped the supracrustal outcrop at a scale of 1:250. This work substantially improves constraints on geologic and geochemical models of these rocks, and supports a >3.83 Ga origin of life.

Supracrustal rocks of Akilia Island are part of the early Archean Itsaq Gneiss Complex of west Greenland, in which bodies of deformed and metamorphosed sediments and lavas occur as meter to >10 km blocks in highly deformed, heterogeneous tonalitic gneisses. The regionally extensive Itsaq Gneiss Complex is assigned an early Archean age based on numerous >3.5 Ga ages of igneous zircons in the tonalitic gneisses. The complex is intruded by younger Archean mafic dikes (Ameralik dikes, ~3.0 Ga) and Proterozoic granitic pegmatites, and experienced at least two middle to late Archean episodes of high-grade metamorphism and deformation.

On Akilia Island, the main supracrustal sequence is exposed on the southwestern peninsula. The outcrop is ~150x200m. Supracrustal lithologies comprise compositionally distinct, mappable layers that are interpreted to correspond to original stratigraphy. Units include mafic amphibolites, ultramafic rocks, and Fe-rich clinopyroxene-quartz rocks, interpreted as metasedimentary banded iron formation. Map relations reveal that Fe-rich metasediment is part of the stratigraphy, rather than a later vein or intrusion. Found within the supracrustals, but not part of the stratigraphy, are from oldest to youngest: tonalitic orthogneiss of the Itsaq Gneiss Complex, Ameralik dikes and late leucocratic pegmatites.

Structural relations of the supracrustals are complex. Three early ductile deformation (D1-D3) events were followed by one brittle deformation (D4). The earliest structures are foliations (S1) parallel to lithologic contacts, with locally preserved upright fold hinges. S1 foliations are isoclinally folded (D2) about a steep axial surface (S2). D3 refolded S2 about a steep NS axial plane (S3). Later brittle deformation is recorded as pegmatitic veins and dikes of presumed Proterozoic age.

A key question about the age of the Akilia supracrustal lithologies centers on the nature of contacts between tonalitic gneisses and the supracrustal rocks. Tonalitic orthogneisses host the supracrustal block, but are also found within it. If the contacts are igneous, then the age of the tonalitic gneisses is a minimum for the supracrustals; if the contacts are structural, then the tonalite age is only a maximum supracrustal age. Ignoring exposures at the margins of the supracrustals, which are ambiguous as to origin, we identified two crosscutting tonalite bodies. When retrodeformed, both require dike-like geometric relations with the host supracrustals. The larger exposure is a 1-10 m wide tonalitic orthogneiss that is deformed by D1-D3 and crosscuts an ultramafic-amphibolite contact in the supracrustal stratigraphy. The second is a small (~15 cm wide) tonalitic orthogneiss vein at a high angle to layering in a mixed amphibolite + ultramafic unit.

Ion microprobe U-Pb geochronology has been carried out on zircons separated from the crosscutting tonalitic orthogneisses. The larger body yielded zircons of 3.85 Ga. High-resolution U-Pb ion microprobe measurements of 21 zircons from the smaller tonalite yielded ages of 3.6 to 3.83 Ga. Of these zircons, a significant number (8/21) have ages that exceed 3.8 Ga and have a tight clustering of ages at 3.83±0.005 Ga. The oldest grains generally have higher Th/U and are interpreted to be the original magmatic population.

In summary, the Fe-rich rocks, in which C isotopic evidence for biologic activity is preserved [2], define a mappable, laterally continuous lithology that is part of a coherent supracrustal stratigraphy. When the complex geometric relations in the supracrustals are retrodeformed, two tonalitic orthogneisses preserve clear crosscutting relations. Measurement of Th/U vs. U-Pb age by depth-profiling reveal that the only possible igneous components of these zircons are the 3.83 Ga cores. All other components are the result of later zircon growth associated with documented high-grade metamorphic events in the early to mid Archean [4]. Thus, our new geologic and geochronologic constraints provide the strongest evidence yet for a sedimentary component to, and a >3.83 Ga age of, the Akilia supracrustals.

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Sedimentary Parameters Controlling Occurrence and Preservation of Microbial Mats in Siliciclastic Depositional Systems

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Shallow-marine, siliciclastic depositional systems are governed by physical sedimentary processes. Mineral precipitation or penecontemporaneous cementation play minor roles. Today, coastal siliciclastic environments may be colonized by a variety of epibenthic, mat-forming cyanobacteria. Studies on microbial mats showed that they are not randomly distributed in modern tidal environments. Distribution and abundancy is mainly function of a particular sedimentary facies. Fine-grained sands composed of "clear" (translucent) quartz particles constitute preferred substrates for cyanobacteria. Mat-builders also favor sites characterized by moderate hydrodynamic flow regimes, which permit biomass enrichment and construction of mat fabrics without lethal burial of mat populations by fine sediments.

A comparable facies relationship can be observed in ancient siliciclastic shelf successions from the terminal Neoproterozoic Nama Group, Namibia. Wrinkle structures that record microbial mats are present but sparsely distributed in mid- to inner shelf sandstones of the Nudaus Formation. The sporadic distribution of these structures reflects both the narrow ecological window that governs mat development and the distinctive taphonomic conditions needed to preserve the structures. These observations caution that statements about changing mat abundance across the Proterozoic-Cambrian boundary must be firmly rooted in paleoenvironmental and taphonomic analysis.

Understanding the factors that influence the formation and preservation of microbial structures in siliciclastic regimes can facilitate exploration for biological signatures in Earth's oldest rocks. Moreover, insofar as these structures can be preserved on bedding surfaces and are not easily mimicked by physical processes, they constitute a set of biological markers that can be searched for on Mars by remotely controlled rovers.

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How Much Did Horizontal Gene Transfer Contribute to Early Evolution?: Quantifying Archaeal Genes in Two Bacterial Lineages

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The occurrence of horizontal gene transfer (HGT) among microbes is well accepted, but the extent to which the transfer of genes has impacted microbial evolution is unclear. Transfers most often occur between closely related organisms, however instances of transfer across domains can be inferred from the analysis of gene sequences. In the case of proton pumping ATPases, the bacterial lineages Deinococcaceae and Spirochaetaceae do not have an F-ATPase like other bacteria, but possess a complete A/V-ATPase typically found in Archaea and Eukaryotes. Members of these lineages also possess archaeal type prolyl tRNA synthetases and malate dehydrogenases (Gogarten et al., 1999). To understand the extent to which these Bacteria have acquired genes from the Archaea, we have used a bioinformatics approach employing whole genome analysis to assess the occurrence of interdomain transfer into these lineages.

Putative open reading frames (ORFs) from the prerelease version of the *Deinococcus radiodurans* genome and ORFs from the available completed genomes of *Borrelia burgdorferi* and *Treponema pallidum* were screened for genes more closely related to archaeal or eukaryotic genes. ORFs were searched against representative reference genomes from each of the three domains using a local version of automated BLAST. Gapped BLAST was run for each ORF against amino acid sequences of ORFs from a single reference genome, using BLASTP with the BLOSUM62 matrix with filtering for low complexity regions. Sequence manipulations using the SEALS package of programs (Walker and Koonin, 1997) greatly facilitated the analysis of the *Treponema* and *Borrelia* genomes. For each BLAST run, only the top hit was reported and the High Score Pair (HSP) or Bit Score was retained in a file. These data were then loaded into an Oracle database or into a Microsoft Excel spreadsheet and ranked according to the difference: $\text{MAX SCORE}_{\text{Archaea, Eukaryotes}} - \text{MAX SCORE}_{\text{Bacteria}}$ where SCORE represents HSP values or Bit Score values depending on the version of BLAST used. Each potential *D. radiodurans* ORF was compared against each of five individual reference genomes: *Saccharomyces cerevisiae, Methanococcus jannaschii, Escherichia coli, Bacillus subtilis,* and *Aquifex aeolicus*. Reference genomes for the *Treponema* and *Borrelia* analyses were the same as those used for *D. radiodurans*, plus *Drosophila melanogaster* and *Archeoglobus fulgidus*. ORFs showing highest matches against archaeal and eukaryotic genes were collected and ranked. For *Deinococcus*, the top ranked hits were the A/V-ATPase catalytic and non-catalytic subunits and the prolyl RS genes. For each top ranking hit, datasets of orthologous amino acid sequences were assembled from the nonredundant protein database of Genbank and analyzed using neighbor joining as implemented in Clustal W and quartet puzzling methods found in PUZZLE.

All trees were assessed for evidence of horizontal gene transfer. Trees scored as positive for HGT were of two varieties-- those that showed typical three domain structure with few bacterial species (including *Borrelia, Treponema* or *Deinococcus*) grouping with Archaea or Eukaryotes, and those that showed disjunct distribution among species represented in the non-redundant database. Disjunct distribution (i.e. with most representatives found among Archaea or Eukaryotes and few homologs among the Bacteria**)** are interpreted to represent genes that have been transferred among the representatives (as opposed to being inherited vertically and lost multiple times). Negative topologies were found among those hits that were not represented in the reference genomes, or among those genes which were highly conserved and showed relatively high bit scores for two or more domains**.** Of the 45 top ranked genes for *Deinococcus*, 20 showed topologies in which *D. radiodurans* homologs clearly group with eukaryotic or archaeal homologs, and seventeen additional trees were found to show probable evidence of horizontal gene transfer (Olendzenski et al., 2000). Top ranked hits for *Borrelia* were the isoleucyl-tRNA synthetase, the V-type ATPase, prolyl-tRNA synthetase, methionyl-tRNA synthetase and lysyl-tRNA synthetase. Of 37 top ranked *Borrelia* ORFs analyzed with phylogenetic methods, 27 showed evidence or potential evidence for horizontal gene transfer between domains.

Compared to the total number of ORFs in the *Deinococcus* and *Borrelia* genomes, those that can be identified as having been acquired from Archaea or Eukaryotes are relatively few (approximately 1-1.5%), suggesting that interdomain transfer is rare. Similar methods need to be employed to assess the potential contribution of intradomain transfer from other Bacterial lineages into these genomes. Additionally, many of the phylogenies showing presence of archaeal genes in the Deinococcaceae or Spirochetes, also include *Chlamydia*, *Chlamydophila*, *Rickettsia*, *Mycobacterium*, and *Streptomyces*. These Bacteria could be candidates for higher occurrence of interdomain gene transfer in comparison to other Bacterial lineages.

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Dissolution Kinetics of Natural Uraninite and the Evolution of Atmospheric Oxygen

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The evolution of the early Earth's atmosphere is a key to understand how the terrestrial biosphere has co-evolved with the Earth's environment. Oxygenic photosynthesis emerged on the Earth sometime before 2.7 Ga (Brocks et al., 1999) and set the stage for the establishment of abundant atmospheric oxygen. However, the current dominant model of atmospheric evolution suggests that the atmospheric oxygen level remained very low until about 2.2 Ga (e.g. Holland, 1984).

The occurrence of detrital uraninite (UO_2) in some 2.9 to 2.2 Ga quartz-pebble conglomerates is the most important evidence of oxygen-poor atmosphere prior to 2.2 Ga. Since uraninite is thermodynamically unstable under an oxygenated environment, the survival of detrital uraninite in river sediments would imply oxygen-poor atmosphere. A quantitative estimate of the pre-2.2 Ga atmospheric oxygen level relies on the dissolution kinetics of uraninite. Based on the kinetic data obtained by Grandstaff (1976), Holland (1984) estimated the pO_2 level of the Archean atmosphere to be less than 0.1 % of the present atmospheric level (PAL). No systematic study on the dissolution rates of natural uraninite has been reported since Grandstaff (1976) but the dissolution rate of synthetic UO_2 glass was recently reported to be about 2 orders of magnitude slower than that obtained by Grandstaff for natural uraninite (e.g. de Pablo et al., 1999).

We constructed a flow-through system to study dissolution rates of natural uraninite under various pH (4 to 8), pCO_2 (1 PAL and 100 PAL) and pO_2 (1 PAL and 0.1 PAL) conditions, in order to better constrain the dissolution kinetics of natural uraninite. The system is similar to the one used by de Pablo et al. (1999) for the dissolution experiments of synthetic UO_2 glass. Natural uraninite samples taken from three localities were powdered to a particle size of 80 to 120 microns, washed, weighed to ~ 0.5 g, and introduced into the reactors. The ionic strength of the leaching solution is set to 0.01 M by $NaClO_4$ and pH is controlled by $HClO_4$ and NaOH. A mixture of N_2, CO_2, and O_2 gas was bubbled through the leaching solution to simulate the Archean atmosphere.

Our data suggest uraninite dissolution rates are slower and less dependent on pH, pO_2 and pCO_2 than those reported by Grandstaff (1976). Dissolution rates of natural uraninite under high pCO_2 (100 PAL) and low pH (4 to 5) solution, a likely condition for the

Archean rainwater, are much slower than that computed from Grandstaff's rate law, indicating detrital uraninite may survive under high pCO_2 Archean atmosphere regardless of pO_2 level. We suggest the occurrence of pre-2.2 Ga placer uraninite deposits may reflect the abundant uraninite-rich source rocks in the area rather than the atmospheric evolution of the early Earth.

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Hydrocarbon Haze as a Source of Low-^{13}C Late Archean Kerogens

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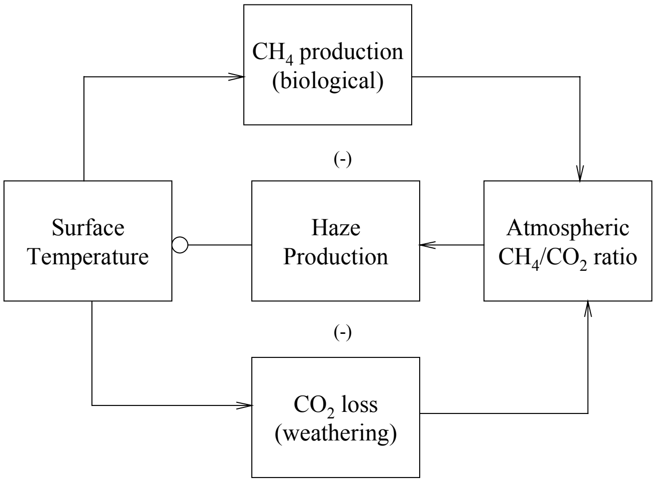
High concentrations of greenhouse gases are required to offset low solar luminosity early in Earth's history. Previous studies suggested high CO_2 concentrations as a solution to the faint young sun paradox (Walker et al., 1981). However, the late Archean atmosphere should have been rich in biologically-generated CH_4 which would have provided additional warming (Pavlov et al., 2000). Calculations based on the thermodynamic energy yield from microbial metabolism predict that methanogens should have converted most of the available H_2 into CH_4, producing an atmospheric CH_4 concentration of about 1000 ppm (Kral et al., 1998; Kasting et al., 2001). An independent line of evidence for high methane abundances comes from the photochemical modeling of methane destruction. If the Archean biota were manufacturing CH_4 at a rate comparable to the modern biota, atmospheric CH_4 mixing ratios of the order of 10^{-3} are predicted (Kasting and Brown, 1998; Pavlov et al., 2001).

Such a high atmospheric CH_4 abundance should have led to CO_2 drawdown by way of a negative feedback loop involving the carbonate-silicate geochemical cycle:



High CH_4 levels would have raised surface temperature, T_s, by increasing the atmospheric greenhouse effect, stimulating biological production of CH_4 and facilitating silicate weathering on the continents, causing atmospheric CO_2 concentrations to decrease. We suggest here that the atmospheric CH_4/CO_2 ratio approached the value of ~1 needed to trigger formation of Titan-like organic haze.

Previous studies predicted CH_4 polymerization and formation of organic haze in the Archean atmosphere (Zahnle, 1986; Kasting and Brown, 1998). However, there was no constraint on how thick this haze layer could have been and at what rate the haze should have been generated. Our calculations show that the optical thickness of the layer could not have been more than 0.6 otherwise, a strong "antigreenhouse effect" would have cooled the surface and killed the methanogens.

Assuming that the Archean surface temperature was not too different from its present value, we have calculated the abundance of hydrocarbon particles in the Archean atmosphere and the rate of their production. The total deposition flux of organic haze particles under those conditions would have been comparable to the modern rate of organic carbon burial.

This haze should have been strongly depleted in ^{13}C relative to ^{12}C because it was formed from isotopically "light" biological methane and was fractionated further during the polymerization process. Hence, it could provide an alternative explanation for the presence of extremely low-^{13}C kerogens in Late Archean sediments.

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The Anoxygenic Filamentous Phototrophs:
(1) Recent Advances in Diversity and Phylogeny
(2) The Place of *Chloroflexus* in High Iron Hot Springs

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The anoxygenic filamentous phototrophs are the deepest branching phototrophs on the Bacterial branch of the tree of life. Until recently, phylogenetic data as complete 16S rDNA sequences were available only for species of the genus *Chloroflexus*. Other uncultured organisms that were thought to be related to *Chloroflexus* were known only from their similar ultrastructural and physiological characteristics. All filamentous phototrophs were lumped into this group with *Chloroflexus* with little or no sequence data to support that association. In the past year sequence data have been obtained in different laboratories for several different filamentous phototrophs. New species descriptions have appeared, new cultures have been isolated, and new physiological data have been produced. We now know that this group of phototrophs is surprisingly large and diverse. All the filamentous phototrophs indeed appear to belong together. Because of their diversity, these organisms may hold as a group important information about the evolution of photosynthesis. Among the recently obtained phylogenetic data are sequences for *Chloronema*, *Oscillochloris*, the hypersaline marine *Chloroflexus*-like organism (MCLO), and the red filamentous "*Roseiflexus castenholzii*." New physiological data are also available for all of these organisms as well as for the red filamentous RCR organisms.

We present a phylogenetic tree showing that substantial differences exist among these organisms but that all the filamentous phototrophs form a cohesive cluster distinct from the chemotrophs in this branch of the tree. There may be a "green line" of descent that includes *Chloroflexus, Oscillochloris, Chloronema*, and the MCLO, all of which have chlorosomes and bacteriochlorophyll *c*. A "red line" of descent may include *Roseiflexus*, *Heliothrix*, and the RCR, all of which lack chlorosomes and bacteriochlorophyll *c* but do have bacteriochlorophyll *a*. The incomplete sequence data for *Heliothrix* and the absence of sequence data for the RCR make this conclusion speculative. All of these organisms except *Chloronema*, are mat-formers. *Chloronema* is planktonic and found in anoxic zones in lakes containing sulfide and reduced iron. *Chloronema*, *Oscillochloris* and the MCLO are mesophiles. The others are moderate thermophiles. Among the interesting features of the diversity of this group are distinctive absorption spectra of the bacteriochlorophylls and diversity in the carbon dioxide fixation pathways. *Chloroflexus* has the unique 3-hydroxypropionate pathway and *Oscillochloris* uses the Calvin Cycle.

Chloroflexus sp. are universal inhabitants of microbial mats in neutral to alkaline hot springs around the world. We have found and cultured a strain from a high iron hot spring mat at 50-54°C. *In situ*, it forms a mat in association with the unicellular cyanobacteria *Synechococcus lividus* and *Cyanothece minnervae*. The mats are very thin and form near the sources of the springs which are anoxic and high in ferrous iron (0.1 mM). Studies of the mats in situ have shown that oxygenic photosynthesis occurs in the light. The mats are substantially lower in oxygen levels and pH, however, than typical *Synechococcus*/*Chloroflexus* mats observed in low iron springs. We are attempting to determine the relationship, if any, between the iron in the springs and the photosynthetic activity in both the cyanobacteria and *Chloroflexus*.

Independent Land Colonization Events by Myriapods and Hexapods During the Neoproterozoic

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The fossil record suggests the macroevolutionary events that led to increased diversity of Metazoan (animal) body plans occurred within a short geological time span known as the Cambrian 'Explosion'. However, recent molecular studies have suggested that animal diversification occurred much earlier. The arthropods figure prominently in the Cambrian Explosion and therefore we chose this group to examine these questions in more detail with new molecular data.

Arthropoda is a diverse animal phylum composed of four living classes whose evolutionary relationships have been controversial. These are Myriapoda (centipedes and millipedes), Hexapoda (insects), Crustacea (crabs, brine shrimp, and water fleas), and Chelicerata (spiders, scorpions, and horseshoe crabs). Traditionally Myriapoda and Hexapoda were considered sister taxa based on characteristics associated with terrestrialization and were placed together in the taxon "Atelocerata". However, new sequence data from two genes, enolase and glyceraldehyde 3-phosphate dehydrogenase, combined with two genes from a previous study, RNA polymerase II (large subunit) and elongation factor 1 alpha, significantly support a Myriapoda-Chelicerata grouping to the exclusion of Hexapoda and suggest a placement of Hexapoda with Crustacea. This indicates that the two separate lineages, Myriapoda and Hexapoda, colonized land independently and that the terrestrial characteristics used to group them are due to convergence. Additionally, these four genes and several others were used for divergence time estimation. The times indicate that these colonizing events took place at about the same time as the Neoproterozoic 'Snowball Earth' episodes, much earlier than the Cambrian Explosion.

Characterization of a Carbonic Anhydrase from an Obligately Chemolithoautotrophic Thermophilic Archaeon

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The atmosphere of earth at the origin of life is thought to be a weakly reduced mixture of CO_2 and N_2 combined with lesser amounts of CO, H_2, and reduced S gases. Experiments by Wachtershauser suggest a chemoautotrophic origin of life in which early organisms could fix CO_2 and CO enzymatically. Carbonic anhydrase, a zinc-containing enzyme catalyzing the reversible hydration of CO_2 ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$), plays a key role in CO_2 fixation in extant organisms. Based on amino acid sequence comparisons, carbonic anhydrases belong to three genetically distinct classes (α, β, and γ) suggesting this enzyme has been invented independently at least three times. Molecular clock analysis indicates that both the β and γ classes have ancient origins suggesting a role for this enzyme in the CO_2 fixation of early organisms. To better understand the role of this enzyme in early organisms, we have both biochemically and structurally characterized a β class carbonic anhydrase (Cab) from the deeply branching obligately chemolithoautotrophic archaeon *Methanobacterium thermoautotrophicum*. This microbe has a high demand for CO_2 in that not only does all of its cellular energy derive from the reduction of CO_2 to methane but also all of its cellular carbon derives from CO_2. Therefore, Cab is expected to play an important role in the growth of *M. thermoautotrophicum* and may have several functions. Of the three known classes of carbonic anhydrase, the β class is the most diverse at the amino acid sequence level. The results of my work confirm that the β class is in fact composed of two subclasses and both biochemical and structural experiments indicate that Cab is the prototype for one of these subclasses. In addition, only five amino acid residues are completely conserved in all β class sequences. Three of these conserved residues serve as ligands for the active site zinc and we have identified potential roles for other two conserved amino acid residues in the catalytic mechanism of β class enzymes.

Molecular Evolution Analysis of Photosynthesis

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Recent initiatives in whole genome sequencing and the collection and successful culturing of new organisms have greatly facilitated the study of the evolution of photosynthesis at the molecular level. The first of these two fronts has resulted in the completion of representative genomes from four of the five known prokaryotic photosynthetic lineages. The second front has lead to the discovery of *Acaryochloris marina*, a cyanobacterial-type marine endosymbiont singular in its use of chlorophyll *d* as a major photopigment. Chlorophyll *d* is structurally and energetically intermediate between chlorophyll *a* and bacteriochlorophyll *a*, a characteristic consistent with a pigment which might have been utilized in the evolutionary transition from anoxygenic to oxygenic photosynthesis.

Our current work is focused on addressing the biochemical and evolutionary aspects of the novel pigment complement in *A. marina* through the sequencing of photosystem and chlorophyll biosynthesis genes. Concurrently, phylogenetic analyses are being carried out using these and newly completed sequences from representative anoxygenic and primitive oxygenic phototrophs with the goal of refining our understanding of early evolutionary events in photosynthesis.

Geochemical and Isotopic Investigation of Paleozoic Paleosols Formed Under Varying Redox Conditions

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The Permo-Pennsylvanian Monongahela Group consists of cyclic freshwater bay, deltaic, and alluvial fan sediments deposited approximately 290 million years ago in the Dunkard basin (SW Pennsylvania, W. Virginia and Ohio). The lacustrine Benwood limestone dominates the deeper parts of the basin. Toward the basin edges, the deposits grade into thin interbedded marly limestones, claystones and shales indicative of a shallower water regime and fluctuating lake levels (Fig. 1). These lake-edge deposits can be divided into two end members: the green (northern) and red (southern) facies [1-3]. In the northern part of the basin, massive limestones are interbedded with green shale and thin (cm-scale) claystones. To the southeast, the limestone beds thin and grade into shales and redbeds. Thick (1-4 m) redbeds, interbedded with clay-shales and limestones, are ubiquitous along the southern rim.

To better understand the geochemistry of ancient freshwater lacustrine sequences and sediments affected by variable redox conditions, we are studying outcrops of red and green units formed along the lake edge, and deeper water lacustrine limestones and claystones from a 50 m core. Units from both facies display evidence of soil-forming processes and/or subaerial exposure. Limestone layers with mudcracks, microkarst, and dolomite suggest cyclic wet and dry periods. Both red and green facies paleosols are characterized by root casts, a diffuse lower contact with the calcareous clay-shale parent material, and a sharp erosional contact with the overlying limestone. Some redbed paleosols also contain calcareous nodules, blocky to columnar ped-like structures, mottling (5R 4/4 and Gley 2 4/5pb), and gilgai, indicative of arid, oxidizing conditions. Green paleosols contain evidence of reducing conditions (such as gley textures, color and mineralogy)

Geochemistry of two redbed paleosol units (Middle Island Creek and England's Run) indicate the net loss of base cations (Ca, P, Mg, K and Na) from the profile and the displacement of more immobile cations (Mn, Fe, and Si) to lower parts of the weathering profile. Preliminary Nd isotope data indicate a narrow range of ε_{Nd} values (-8 to -10) for the silicate fraction of lacustrine calcareous shales and paleosol silicates, suggesting derivation from a common, constant sediment source. $^{143}Nd/^{144}Nd$ *vs.* $^{147}Sm/^{144}Nd$ and $^{87}Rb/^{86}Sr$ *vs.* $^{87}Sr/^{86}Sr$ display linear correlations consistent with the derivation of both lake detritus and paleosols from isotopically homogeneous Permo-Pennsylvanian sediments that were fractionated with respect to REE and Rb/Sr during sedimentary or pedogenic processes.

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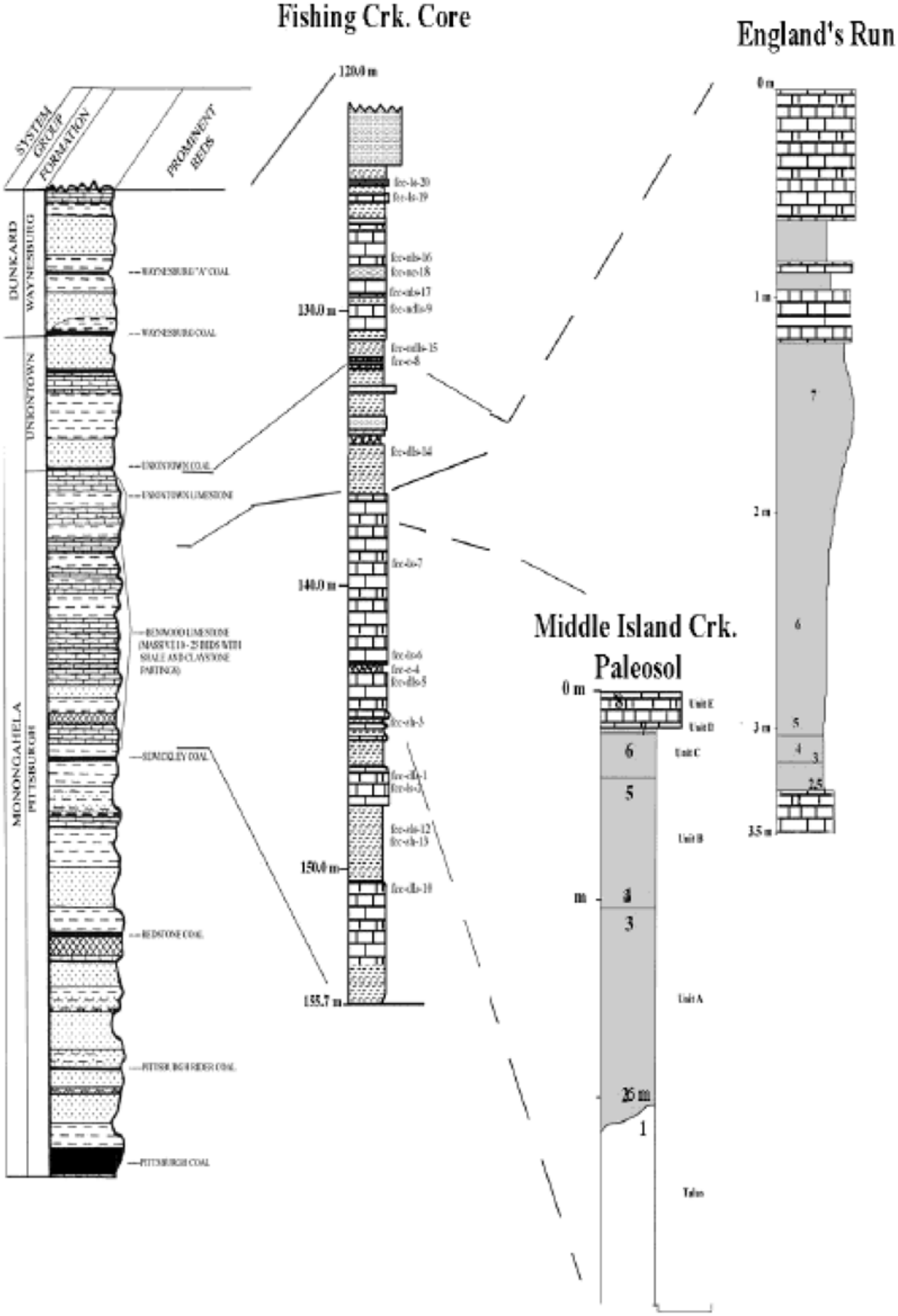

Fig. 1 (after C. Kairies, 2000)

Archean Sulfates from Western Australia: Implications for Earth's Early Atmosphere and Ocean

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Early Archean sedimentary sulfates (barites) from Africa, Australia, and India are only slightly enriched in the heavy isotope of sulfur in comparison with contemporaneous sulfides. This small separation of oxidized and reduced reservoirs has long been used as evidence for and against the activity of sulfate-reducing bacteria prior to about 2.5-2.7 Ga ago. However, it is now clear that the accelerated rates of bacterial sulfate reduction together with higher ambient temperatures and closed-system settings advocated by Ohmoto and others cannot easily explain the limited isotopic variability and slight ^{34}S enrichment of Archean sulfates (Canfield et al., 2000). The recent discovery of sizeable, mass-independent fractionations of all four stable isotopes of sulfur in early Archean sulfates from Africa and Australia (Farquhar et al., 2000), confirming earlier evidence from India, provides a new opportunity for understanding the Archean sulfur cycle.

Pioneering work on bedded barites from the North Pole area of Western Australia (Lambert et al., 1978; Buick and Dunlop, 1990) indicated that they are diagenetic replacements of evaporitic gypsum crystals that had grown on or just below the Archean sea floor. This interpretation was based on interfacial angles measured with a universal stage on relatively few crystals. In order to improve the crystallographic database, X-ray computerized tomography (CT) was used to image in situ barite crystals. At the operating energy levels, barite attenuates X-rays significantly more than metallic iron, so the contrast between the barite crystals and the quartz matrix is profound. Analysis of the processed images, casts of natural external molds of barite crystals, and large thin sections using polarized light, conclusively demonstrates that the original mineralogy was barite, not gypsum. A close genetic link between the bedded barites and syndepositional barite dikes is provided by plots of all four stable isotopes of sulfur which form arrays that parallel terrestrial mass fraction lines but have y-axis intercepts significantly far from zero. This is excellent evidence that the hydrothermal fluids involved in dike production (Nijman et al., 1999) were cycling sulfates from the same source as the bedded barites. The non-zero intercepts are indicative of sizeable mass-independent fractionations of the sulfate. As these effects are only known to be produced by atmospheric (gas-phase) processes in terrestrial materials, it is likely that the barites were formed from sulfate sulfur that had participated in atmospheric reactions. A corollary is that the Archean oceans may not have contained much sulfate.

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Neodymium Isotope Investigation of a Precambrian Weathering Profile: Hokkalampi Paleosol, North Karelia, Eastern Finland

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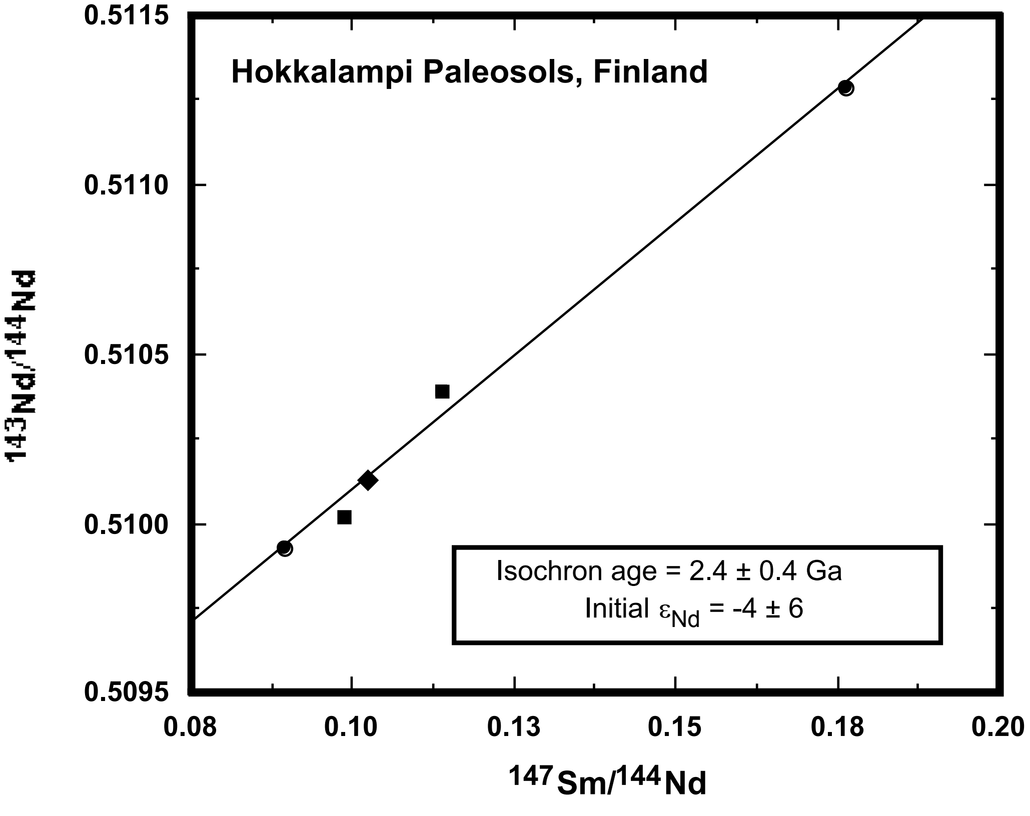
Paleosols, preserved ancient soils, are significant to the study of past life, climate, and landscapes on Earth and serve as the primary analogues for the development of life on other planets. By preserving chemical products of atmospheric-mineral interaction, Precambrian paleosols are also key to understanding the past chemistry and evolution of the Earth's atmosphere. However, many challenges inhibit the interpretation of the Precambrian soil record including difficulty constraining ages, overprinting by thermal events, and deformation of soil textures and structures [1]. To better understand the diagenetic and metamorphic processes that have obscured the ancient soil record, we have begun a neodymium (Nd) isotope study of the Hokkalampi paleosol in eastern Finland. Nd isotopes have proven useful in previous study of the 3.0 Ga Steep Rock paleosol, which was overprinted by a 2.7 Ga greenschist metamorphic event. The thermal event did not mask the geochemical signature of the Nd isotopic system. The measurements formed an apparent isochron that could date the age of soil formation [2].

The Hokkalampi paleosol is of particular interest because it formed between 2.5 Ga and 2.1 Ga [3] during the proposed rise of atmospheric oxygen [4]. Previous work by Marmo [3] established geochemical evidence that the Hokkalampi area contains a widespread paleosol. The weathering profile formed on Archean basement and glacioigenic material and is unconformably overlain by aluminum, quartz, and/or hematite rich metasedimentary rocks [3]. The age of the paleosol is constrained between the Archean basement (3.1-2.5 Ga; [5]) and the 2.1 Ga mafic rock which intrudes the overlying metasediments. The profile grades upward from granitoid parent to carbonate bearing, quartz-feldspar sericite (partially altered parent) to quartz-sericite schist (weathering profile). The paleosol, produced by intense chemical weathering, ranges in thickness from 15 meters at the paleotopographic high to 80 meters at the paleotopographic low. The weathering profile ranges from oxidized, iron-enriched areas to reduced, iron-poor areas. The paleosol was altered by a greenschist metamorphic event 1.9 Ga during the Svecofennian Orogeny [3].

Outcrop and drill core samples were collected from two localities: Nuutilanvaara and Paukkayanvaara. Both paleosol locations formed over Archean basement. However, Nuutilanvaara contains oxidized paleosol with iron enrichment and is interpreted to be above the paleo-water table; Paukkayanvaara contains reduced paleosol with iron depletion and is interpreted to be below the paleo-water table. Whole rock Sm-Nd measurements were carried out on representative zones from each locality, including parent rock, quartz-feldspar sericite (partially altered parent), and quartz-sericite schist (weathering profile). When ^{143}Nd/^{144}Nd and ^{147}Sm/^{144}Nd data from both localities are plotted together, they form a linear array corresponding to an apparent age of 2.4 ± 0.4 Ga (Fig. 1). If the correlation indeed represents a crude isochron, two possible explanations are suggested: (1) The age corresponds to timing of basement formation, and the whole rock samples represent reworked, fractionated basement material; or (2) pedogenic processes fractionated Sm/Nd, the age represents the time of pedogenesis. In either case, the Nd isotope systematics indicate that the parent material for both profiles is derived from the same, relatively uniform source. This makes the Hokkalampi profile an excellent candidate to compare early Proterozoic weathering processes under locally varying redox conditions.



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Shallow to Deep Water Transitions on Early Archean Subsiding Platforms, Barberton Greenstone Belt, South Africa

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Sediments from the early Archean were commonly extensively and syndepositionally silicified. In areas of high organic burial and low clastic input, typical resultant carbonaceous facies are massive black cherts, black and white banded cherts, and banded ferruginous cherts. While these chert types are recognized in many early Archean units, their significance for environmental interpretation is unclear.

Two sections were measured through carbonaceous cherts in the uppermost Mendon and Kromberg formations. The detrital content of each section was extremely low; this is interpreted to indicate deposition on platforms. The first section (MBC1), in the uppermost Mendon, progresses from an erosional contact with the underlying Msauli Chert to banded ferruginous cherts and flat-laminated ash at the top. The second section (BRC1), in the uppermost Kromberg, progresses from silicified evaporites at the base to banded ferruginous cherts at the top. Both sections show characteristic progressions from massive or current/wave structured black cherts, through black and white banded cherts showing progressively thinner and more laterally continuous bands, to banded ferruginous cherts. It is possible that this progression generally characterizes shallow to deep water transitions in clastic-poor, organic-rich, silicified sediments from the early Archean, and that these sections record changing environments on subsiding platforms.

Ongoing work on these sections includes a petrographic classification of carbonaceous grain types, measurement of bulk elemental compositions, and measurement of carbon isotopic compositions of bulk kerogen, carbonate, and individual carbonaceous grains. These analyses should allow description of changing microbial communities and redox conditions accompanying changing water depth on these platforms.

The Combined Detection of Morphological and Molecular Biomarkers: Implications for Astrobiology

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Introduction: Experience gathered by previous researchers during their hunt for evidence of early Earth life has shown the complexity in interpreting observations of possible microfossils and to establish the evidence to be positive [1]. Similarly, the still-simmering controversy on the nature of the nano-structures in Martian meteorite ALH84001 described by McKay *et al.* (1996) [2] emphasizes the difficulties of conclusively identifying those structures as (a) fossilized bacterial cells and (b) establish their indigeneity. A better understanding of biological signatures in rocks is needed in order to identify traces of microbial life, which include morphological, mineralogical and chemical traces. It is thus considered crucial to tackle the problems emerging in the search for evidence of early life on Earth and in exopaleontological research with a multi-disciplinary approach [1, 3]. With this is mind we applied surface sensitive Time of Flight – Secondary Ion Mass Spectroscopy (ToF-SIMS) to a previously described 25 m.y. old fossil bacterial biofilm [4, 5]. This technique allows *in situ* analysis with high mass resolution as well as molecular imaging of micron sized structures. As no extraction or derivatisation of the sample is required for ToF-SIMS analysis, electron microscopical investigation of the same samples subsequent to analysis is possible, thus allowing the combination of molecular and morphological biomarkers.

Materials and Methods: The analysed fossil bacterial biofilms were associated with macrofossils from volcanoclastic lacustrine sediments from the Upper Oligocene Enspel formation (Germany). Preliminary scanning electron microscopy (SEM) studies have shown that a fossil structure interpreted as a coprolite [5] purely consisted of fossilized bacterial biofilm. For ToF-SIMS investigation small particles were taken from the fossil biofilm and mounted onto Au-coated In-foil and analysed in a Phi Evans T-2000 TRIFT system. The ToF-SIMS analysed samples were Au/Pd-sputter coated and imaged using a Philips XL40 Field Emission Gun SEM (FEG-SEM).

Results and Discussion: ToF-SIMS analysis of the organic rich fossil biofilm (TOC 29%) in the 0-100 Dalton (Da) range showed significant amounts of inorganic species, confirming the results obtained previously by EDX analysis [5], clearly showing the bacterial fossils to be mineralised. ToF-SIMS furthermore revealed the presence of a variety of low- and high-mass organic molecules and fragments thereof. These include peaks indicative of alkenes and alkanes [6], aromatic organic species and the polycyclic aromatic hydrocarbon naphthalene [7]. More tentatively, peaks indicative of alkyl pyrroles and pyridyl-CH_2 were identified [6]. Other peaks of interest include peaks indicative of $C_nH_{2n}O_2$ and $C_nH_{2n-2}O_2$, which according to their general formula would suggest the presence of both saturated and unsaturated fatty acids although further *in situ* derivatisation experiments and GC-MS (Gas Chromatography MS) need to be applied to verify this beyond doubt. Furthermore, peaks at *m/z* 370, 384, 398, 412, 426, 440, 454 and 468 were identified, which indicate the potential presence of bacterial hopanes [8], a class of biomarkers indicative of bacteria. The main diagnostic peak for this group of chemicals is the fragment at *m/z* 191.18. Our studies conducted on purified hopane standards have shown that in the high-mass resolution mode differentiation of this diagnostic hopane peak and polyethylene at *m/z* 191.05 is possible [9]. However, the spectra discussed here were collected in the lower resolution mapping mode, therefore this differentiation was not possible. The centroids of the possible hopane peaks obtained on the fossil biofilms are well within the range associated with bacterial hopanes. There is a strong possibility therefore that hopanoids may be associated with the fossil bacterial cells. Due to the non-destructive nature of ToF-SIMS, analysed samples can be studied using SEM, thus allowing the combination of morphological and molecular biomarkers.

Conclusion: Subsequent SEM analysis of the ToF-SIMS analysed samples confirmed that the analysed material purely consists of fossil bacterial cells. This is thus the first successful effort to demonstrate the combination of spectral and morphological biomarkers. The advantages of highly sensitive non-destructive *in situ* analysis techniques for biomarker detection are invaluable, particularly with respect to envisaged Mars sample return missions, as it may allow us to identify remains and traces of former microbial life in both ancient terrestrial and extraterrestrial materials. This technique may prove particularly useful in the quest for extraterrestrial life with respect to precious extraterrestrial materials, as minute quantities are sufficient to conduct analysis.

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Earth's Orbital History Revisited

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Orbital calculations are increasingly being used, in conjunction with sedimentary and geochemical data, to analyze environmental evolution on planetary scales. In order to better understand the history of the inner Solar System over timescales of hundreds of millions of years, we have carried out a number of accurate, long-term, numerical simulations of the orbits of the nine major planets using physical models with increasing complexity. In this work, equations of motions are directly integrated by a Stormer-Cowell multi-step scheme which is optimized for reduced round-off errors. We gradually refine the physical models to include corrections required by general relativity, the finite size of the lunar orbit, and the solar gravitational quadrupole moment. In one case, the Earth-Moon system was resolved as two separate bodies and the results were compared to those based on analytically averaging the lunar orbit. A better analytical averaging model is obtained by means of these comparisons.

The computed orbits are in good agreement with those of previous studies for the past five million years but not for earlier times. Chaos in the motion of the inner planets limits the validity of the computations beyond 50 million years. Nevertheless, we detect a number of significant transitions at earlier times that involve changes in the precession of the orbits of Earth and Mars. One in particular, about 65 million years ago, is associated with clearly discernible, macroscopic changes in the evolution of Mercury's orbit, suggesting the possibility of a dramatic shift in the dynamics of the Solar System at that time. This dynamical transition could have been responsible for significant perturbations within the asteroid belt and that it corresponds, approximately, to the Cretaceous-Tertiary boundary.

Development of Cyanobacterial Mats on Soil Surface under an Oxygenated Atmosphere 2.6 Gya Ago at Schagen, Eastern Transvaal, South Africa

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The 2.6 Ga Schagen paleosol developed on a >2.7 Ga ultramafic rock (serpentinized dunite) and was overlain by a 2.56 Ga marine-quartzite unit. They were subjected to the green-schist facies metamorphism 2.06 Ga. The present thickness of the paleosol is about 17 m. This paleosol, characterized by high contents of organic carbon (up to 1.2 wt%) as well as of calcite and dolomite, is similar to modern "calcretes" and "dolocretes" that developed under (semi) arid climate. Another noteworthy characteristic of the paleosol is an association of organic matter and ferric-bearing silicates (ferri-stilpnomelane), especially at the top. Aluminous minerals (e.g., sericite) are generally rare because the parental rock is poor in Al.

Based on the mineralogical characteristics, the Schagen paleosol is divisible into six zones. From top to bottom, they are: (I) quartz-rich zone, (II) calcite-rich zone, (III) talc-rich zone, (IV) dolomite-rich zone, (V) calcite-rich zone, and (VI) carbonated-rich serpentinized zone. Organic C is mostly accumulated with fine-grained clay minerals, forming seams in the upper 5 m of this section (i.e., zones I – III). In the quartz-rich zone I, organic-rich seams occur on fine-grained layers of detrital grains of quartz; the organic-rich seams are sometimes traceable over a few centimeters in thin sections. Organic matter also occurs along grain boundaries of quartz, carbonate and talc. Such occurrences of organic matter suggest that it is a remnant of microbial mats developed on soil surface. This suggestion is supported by the finding that the organic C contents, ranging from 0.2 to 1.2 wt%, positively correlate with the contents of bio-essential elements, such as P and N. The $\delta^{13}C$ values of the organic C range from –17.4 to –14.3‰. These values are significantly different from those of organic matter in the overlain marine sediment (i.e., ~ -33‰), but are similar to those of modern, cyanobacteria-dominated microbial mats developed on land. The carbon isotopic fractionation between the organic matter and the contemporaneous atmospheric CO_2 is estimated to be about –12‰. This value is consistent with the fractionation during CO_2-fixation by oxygenic cyanobacteria via the Calvin cycle. The possibility of other types of photoautotrophs (e.g., purple sulfur bacteria that use H_2S as an electron donor instead of H_2O) as candidates for the microbial mats can be ruled out because of the virtual absence of sulfur in this soil profile.

The organic matter at Schagen is closely associated with Fe-bearing minerals, particularly ferric-bearing mineral, such as ferri-stilpnomelane, in the upper part of the paleosol. The precursor minerals of ferri-stilpnomalane were probably goethite, as the Fe^{3+} source, and kaolinite as the Al source. The presence of goethite suggests that the soil developed under an oxic atmosphere.

Significant losses of Fe from the lower paleosol section, especially from zones II and III, by organic acids generated from the decay of bacterial mats are suggested from the following observations: (a) significant decreases in Fe^{3+}/Ti and $\sum$Fe/Ti ratios compared to the parental rock; (b) decreasing contents of organic carbon downward; (c) occurrences of organic carbon: primarily as fine-grained dissemination along grain boundaries; (d) lower $\delta^{13}C$ values (by ~ 2 ‰) of carbonates in zones II - IV, compared to those in organic-C poor carbonates of zones V - VI. A loss of Fe from paleosol has been considered by previous researchers as an important line of evidence for a reducing atmosphere prior to 2.2 Ga (e.g., Holland, 1994). However, our observations of the 2.6 Ga Schagen paleosols suggest that the mechanism of Fe loss during soil formation has been the same since at least 2.6 Ga, i.e., by organic acids produced by the decay of soil organisms, rather than by organic-free, H_2-rich soil water. A loss of Fe from a paleosol, regardless of whether it is pre- or post-2.2 Ga in age, may be an excellent indicator of biological activity on the land surface.

Organic Carbon, S, Mo, U, and V in the Archean and Paleoproterozoic Black Shales

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Holland (1984) suggests that concentration relationships between organic carbon (OC) and some redox-sensitive elements (Mo, U, V, etc.) in marine shales are useful indicators of the atmospheric oxygen level. According to his theory, an oxic atmosphere promotes the dissolution of some redox-sensitive elements from rocks during weathering, increasing the concentrations of these elements in the ocean water. Consequently, the marine shales deposited under an oxic atmosphere are expected to have higher contents of these elements and positive correlations with the OC contents compared to those deposited under an anoxic atmosphere. However, the Mo-U-V-C-S relationships in modern marine sediments do not fit into such a simple prediction. We have also found that the Mo-U-V-C-S relationships in shales of Archean and Proterozoic age are essentially the same as those in the Phanerozoic shales.

We have analyzed more than 100 samples of black shale from South Africa and Australia that are Archean and Paleoproterozoic in age. They are all from deep drill cores, thus free of the modern weathering effects. They include black shale from the ~3.4 Ga Fig Tree, the ~2.9 Ga West Rand, the ~2.7 Ga Fortescue, the ~2.6 Ga Platberg, the ~2.5 Ga Wolkberg, the ~2.5 Ga Chuniespoort, the ~2.5 Ga Hamersley, and the ~2.4 Ga Pretoria Groups. For comparison, similar data sets from more than 100 samples are collected from literature on OC-rich shales and sediments of many different ages.

The compositions of most Archean-Paleoproterozoic black shales are essentially identical to those of the PAAS (post-Archean Australian average shale) and of some Cretaceous black shales: OC = 0 - 1 wt. %, S = 0 - 1 wt. %, Mo = 0 - 5 ppm, U = 0 - 3 ppm (some as high as 7 ppm) and V = 10 - 200 ppm. The molybdenum and OC content of most of the Archean-Paleoproterozoic shales are also comparable to those of some modern marine sediments in the Sannich Inlet and the Oslo Fjord. High Mo content are typically associated with high sulfide-S content (e.g., >4 wt. %) in the ~2.5 Ga Hamersley Group samples, as well as in the Phanerozoic, and modern sediments. These data suggest that the geochemical cycles of the redox-sensitive elements have been essentially the same since the Archean, specifically the Mo content of seawater has been essentially the same and the concentration mechanisms have been the same. This suggestion is substantiated from a theoretical consideration (below).

Most of molybdenum in sedimentary, metamorphic and igneous rocks occurs as a trace constituent of pyrite and minor molybdenite. Therefore, the weathering flux of Mo during soil formation can be quantitatively evaluated using the theoretical model of oxidation of kerogen in soil (Lasaga and Ohmoto, in review) and experimental data on the kinetics of pyrite oxidation (e.g., Kamei and Ohmoto, 2000). Our calculations suggest that all pyrite grains less than 1 cm^3 in size will be completely oxidized and all Mo will be leached out from a soil zone in less than 10,000 years, if the atmospheric pO_2 level is higher than 10^{-6} atm (= 0.0005 % PAL). This pO_2 value will be lowered if the grain size of pyrite is smaller and/or the residence time of pyrite in a soil zone is longer. Therefore, in contrast to a popular view, the riverine flux of Mo into ocean has most likely been constant since Archean. The enhanced Mo enrichments in shales have occurred only in local or regional basins with an euxinic (and sulfidic) bottom water body. Our study suggests that the Phanerozoic-style redox structure of the ocean, i.e., generally oxic ocean with localized anoxic/euxinic basins where bacterial sulfate reduction is active, may have already developed in the Archean.

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Maximum Likelihood Mapping as Tool for Comparative Genome Analyses

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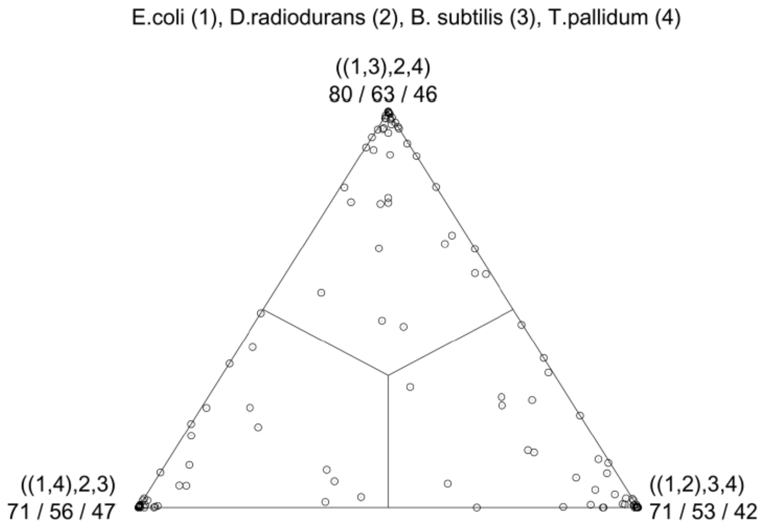
Comparative genome analyses confirm that horizontal gene transfer (HGT) played an important role in shaping microbial genomes. In addition to genes under sporadic selection, HGT also affects housekeeping genes (e.g., archaeal-type ATPsynthase transferred to Deinococcaceae and Spirochetes) and functions involved in information processing (e.g., archaeal lysyl-tRNA synthase in Rickettsia and Spirochetes), and even ribosomal RNA encoding genes (cf., Olendzenski et al., 2000, Woese et al., 2000, Yap et al., 1999). However, the association of tree-like diagrams and evolution is so strongly engrained in evolutionary theory that few alternatives have been explored. Here we describe a tool that provides an assessment and graphic illustration of the mosaic nature of microbial genomes.

The focus on tree-like behavior has been a limitation of previous methods to analyze the phylogenetic information contained in molecular data. Split trees (Bandelt et al., 1992) and maximum likelihood mapping (Strimmer et al., 1997) are two of the few methods developed to assess the non-tree like nature of molecular data. Focusing on the analyses of quartets of molecular sequences, maximum likelihood mapping transforms the three-di-mensional data for each sequence quartet into an easily visualized diagram depicting the phylogenetic content. This approach was designed to gauge the phylogenetic information contained in a multiple sequence alignment; however, it can be easily adapted to the analyses of all quartets of orthologous genes found in four genomes. We have automated the assembly and analyses of these quartets of orthologues given the selection of four genomes, and have analyzed several genome quartets using this maximum likelihood mapping approach.

The possible phylogenetic relationships between four homologous sequences are three unrooted trees. If L_1 , L_2 , L_3 are the three maximum likelihoods for the data under the three possible tree topologies, and if one assumes an equal prior probability for each of the topologies, then the posterior probability pi for topology i (i=1,2,3) is $p_i = Li /(L_1 + L_2 + L_3)$. The probabilities for the three tree topologies add up to one; therefore, the three dimensional data for each sequence quartet can be depicted in a two dimensional simplex (see figure). Given a quartet of genomes, we used blast searches of every ORF against the other three genomes to define quartets of putative orthologues. Our appr oach to define orthologues is similar to the NCBI's definition of COGs (clusters of orthologous groups of proteins, Tatusov et al., 2000); however, Tatusov et al. require reciprocal matches for only 3 genomes, whereas our analyses requires reciprocal matches between the four genomes analyzed. An example of an ml mapping analysis of four genomes is depicted in the figure.



Example of a maximum likelihood mapping analysis of four genomes. Each dot represents the probabilities for the three possible tree topologies plotted in a barycentric coordinate system given one set of orthologous genes, i.e., the distances of a point to the three sides represents the three probabilities. The respective tree topologies are denoted in parenthesis notation in the corners. The numbers below give numbers of quartets of orthologues that support the topology at the majority, .9 and .99 probability level respectively.

In many instances we find nearly equal numbers of quartets supporting the three possible topologies. Furthermore, the strength of support of individual quartets for the different topologies is surprisingly strong. For the largest part these data do not appear to reflect ill resolved phylogenies, rather, each of the three possible topologies has surprisingly strong support by a large fraction of the analyzed quartets.

We will present data comparing the ML-mapping approach to a similar approach using bootstrap probabilities, and analyses using mitochondrial genomes, which presumably were inherited as a single linkage group, as test cases. Our analyses of the genome of the cyanobacterium *Synechocystis* sp. indicate that a large part of the cyanobacterial genome is related to that of low GC Gram positives, confirming an analysis of the evolution of the chlorophyll biosynthetic pathway (Xiong et al., 2000). Our analyses suggest that the interkingdom relationships among prokaryotes cannot be depicted by the tree like evolution of a core of conserved and little transferred genes, rather the prokaryotic genomes are mosaic where different parts have different evolutionary histories.

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